THE UNIVERSITY OF ALBERTA

TOWARD THE SYNTHESIS OF TETRACYCLINE RING-A ANALOGS

BY

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled TOWARD THE SYNTHESIS OF TETRACYCLINE RING-A ANALOGS submitted by ALLAN LOK CHANG MAK in partial fulfilment of the requirements for the degree of Master of Science in Pharmaceutical Chemistry.

IN MEMORIAM

Darlene Jean McAllister

May 5, 1957 — March 29, 1978

ABSTRACT

Studies directed at the synthesis of the ring-A analog 122 are described. The carboethoxycyclohexane-1,3-dione 126 was converted to the ethyl urethan 130, via the hydrazide 129. An improved synthesis of 4-benzamido-cyclohexane-1,3-dione (114), from methyl vinyl ketone (131) and ethyl nitroacetate (132) is reported. Reaction of dimedone (96) with α-chloroacetyl isocyanate afforded a 2-oxazolin-4-one derivative 149, as did the identical reaction on 114 to give 154. This reaction provides a novel approach to these potentially therapeutically important 2-oxazolin-4-ones. Other ring-A analogs synthesized in this study include compounds 138, 144 and 153.

ACKNOWLEDGEMENTS

I would like to express my indebtedness and appreciation for the interest, assistance and constant encouragements of my research supervisor Dr. R.E. Moskalyk.

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INTRODUCTION

The electroreduction of the tetracycline antibiotics has elicited much interest from a number of workers in the area. Doskocil and Vondracek[1] were the first to investigate the reduction of chlortetracycline (1) using polarography. Krestynova-Telupilova et al.[2] demonstrated that the reduction of oxytetracycline (2) was similar to that described for chlortetracycline. In both instances, the total reduction appeared to take place in two two-electron waves which the authors attributed to the reduction of the two conjugated carbonyl systems.

Correlation of the reduction waves with specific functional groups in the molecule was made by Doskocilova[3] from polarographic data acquired on various decomposition products of the tetracyclines. He concluded that the only simple reducible system common to all compounds was the double bond in ring A which is conjugated with the C-1 carbonyl. The more positive reduction potential was assigned to this moiety. The more negative reduction potential was assigned to the second conjugated carbonyl at the C-11 position. All the conclusions drawn by Doskocilova were on the basis of polarographic wave analysis and no attempt was made to identify products or to determine the number of electrons transferred.

Caplis[4] undertook an investigation of the polarographic reduction of several tetracyclines in aqueous media with the view to establishing a mechanism of reduction and also to correlating a specific wave to a definite functional group. He obtained a system of waves that became increasingly complex as the pH was lowered. At pH 4.1, Caplis presented evidence to indicate that the wave at -1.0 V was due to reduction of the hydrogen atom on the tertiary amine salt at C-4. He concluded his studies in aqueous media by stating that the total limiting current and half-wave potentials

were pH dependent and related in some manner to the second pKa value for tetracycline and the A-ring. He further stated that the first reduction wave observed at more positive potential was complex at acid pH and involved more than a single reduction on the molecule. The reduction of the hydrogen from the tertiary amine salt was, in part, responsible for this wave. The total reduction at acid pH probably involved a transfer of five electrons.

This rather vague and inconclusive statement prompted work in this laboratory[5] designed to seek a more definite assignment of the polarographic waves to specific functional groups on the molecule. One aspect of the above mentioned work was the investigation of the polarographic behavior of simpler analogs of the tetracyclines. Attempted synthesis of the ring A analog (122) (see p.43) by the only procedure available in the literature[6] failed to yield the desired product. Correspondence with the author revealed that a sample of the compound was not available. Suggestions from the author for dealing with strategic steps in the synthesis failed to resolve the problem. In view of the problems experienced in this laboratory[7] with the published method, it was decided to abandon further attempts and instead work on a new and different approach to this compound (122). The purpose of this study, therefore, was to investigate synthetic routes that might lead to the preparation of this compound and/or other ring A analogs of the tetracyclines.

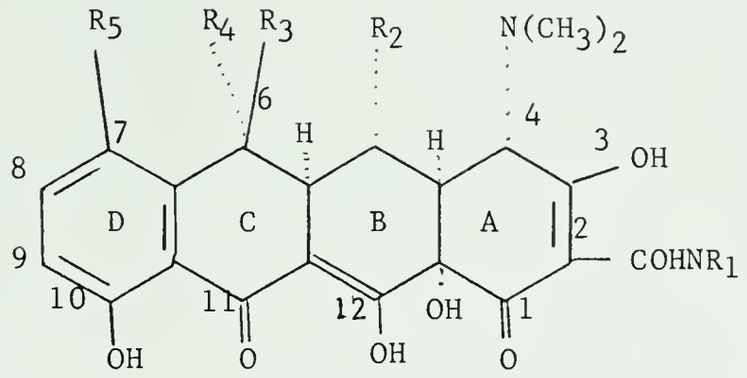


Table I: Chemotherapeutically important tetracyclines

	R_1	R_2	R_3	R_4	R_5	Generic Name
(1)	H	H	OH	CH_3	Cl	chlortetracycline
(2)	H	OH	OH	CH_3	H	oxytetracycline
(3)	H	H	OH	CH_3	H	tetracycline
(4)	H	H	OH	H	Cl	demethylchortetracycline
(5)	$-CH_2-N$ 	H	OH	CH_3	H	rolitetracycline
(6)	H	OH	$=CH_2$		H	methacycline
(7)	H	OH	H	CH_3	H	doxycycline
(8)	H	H	H	H	$N(CH_3)_2$	minocycline

SURVEY OF LITERATURE

1. General

Since their introduction as therapeutically important antibiotics, the tetracyclines have been the object of numerous studies and a number of review articles have been devoted to their chemistry[8,9,10], biogenesis[11], general pharmacological properties[12], mode of action[13] and structure-activity relationships[9,14]. This literature review will be restricted to a survey of the synthetic analogs of the tetracyclines which have been reported in the literature and, where known, their relative antibacterial properties.

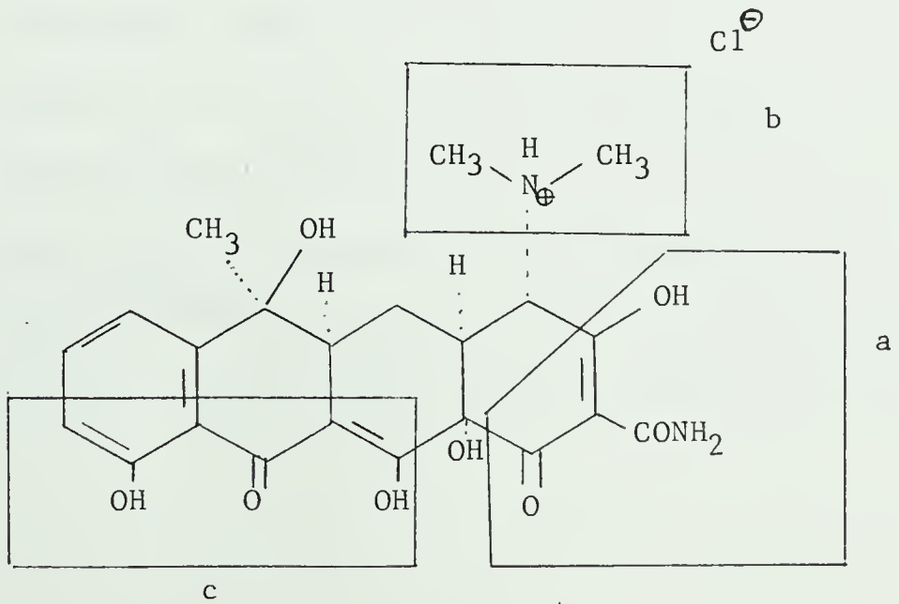
The first tetracycline discovered was chortetracycline (1), isolated by Duggar in 1948 from the culture filtrate of Streptomyces aureofaciens[15]. Oxytetracycline (2) was isolated by Finlay[16] in 1950 from Streptomyces rimosus. In 1953 tetracycline (3) itself, which can also be obtained by hydrogenolysis of chlortetracycline[17,18], was reported to be produced by Streptomyces aureofaciens and by other Streptomycetes[19].

2. Structural Elucidation and Stereochemistry

Oxytetracycline was the first tetracycline whose structure was completely elucidated by Woodward[20] through a series of elaborate degradative studies. X-ray analysis on the relative configuration of the five asymmetric carbon atoms in chlortetracycline hydrochloride was reported[21] in 1959, and was followed by a refined model by Donohue[22] in 1963, confirming structure 1. Dobrynin[23] also established that structure 1 represented the absolute configuration of the compound. Since then the studies of the stereochemical attributes of the tetracycline ring system have included X-ray analysis on the free base[24,25], nmr studies[26], and circular dichroism[27,28]. The structures of the various tetracyclines as depicted in Table I are those generally accepted by chemists in this field.

3. Characteristic Physical and Chemical Properties

Tetracycline hydrochlorides are orange-yellow, odorless, bitter, light-sensitive crystalline compounds. The natural members are sparingly soluble (ca. 1 mg/ml) in water at the physiological pH region[10], and show acidic behavior due to the tricarbonyl methane system in ring A and to the phenolic β-diketone system (C-10, C-11, and C-12). Together with the basic dimethylamino group at C-4 these substituents make the tetracyclines amphoteric compounds. The measured pKa values of, for example, tetracycline hydrochloride in aqueous solution are 3.3, 7.7 and 9.7 and the isoelectric point is 4.8[29,30]. On the basis of a comparison of the pKa values of tetracyclines 1, 2 and 3 with those of tetracycline methiodide (the quaternary methylammonium salt), Leeson, Krueger, and Nash[31] proposed that the first acidity constant was attributable to the tricarbonyl methane system a, while the second and the third acidity constants were due to the phenolic diketone moiety c and the ammonium cation b, respectively (3, HCl). Garrett's investigation[32] of the effect of the dielectric constant on the pKa values of tetracyclines in DMF-water mixtures supported these assignments.



3, HCl

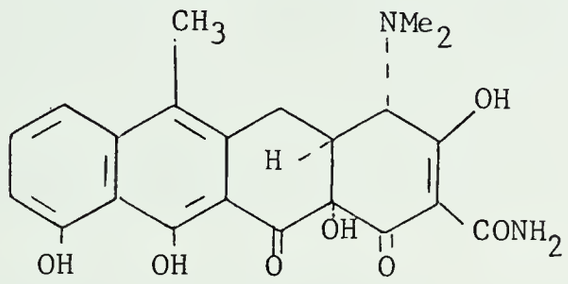
All natural tetracyclines give UV spectra that are highly characteristic and remarkably similar in shape and intensity. The BCD chromophore absorbs at 360, 320, 285, and 225 nm, while the ring A chromophore contributes the 262 nm band due to the π to π^* transitions of the tricarbonyl methane system[33]. The band at 275 nm is of a composite nature[34].

Tetracyclines undergo degradations and rearrangements under acidic and alkaline conditions[9,13]. These compounds are also highly reactive toward reducing agents. Most C-6 hydroxy tetracyclines (for example, tetracycline (3)) are dehydrated at C-5a-6 by acids with concomitant aromatization of ring C to form anhydrotetracyclines, such as 9. In the presence of mild base (5% $NaHCO_3$ solution) the tetracyclines isomerize to isotetracyclines; chlortetracycline (1) is particularly labile and forms isochlortetracycline (10) by simply warming at a pH of 7.5. Anhydrotetracyclines show weak antibacterial activity which may come from a different mode of action[35]. Isotetracyclines have no significant activity[8].

6-Deoxytetracyclines differ from natural tetracyclines in their higher stability toward acids and bases (since the 6-hydroxy group which is responsible for the acid and alkaline lability is missing).

The dimethylamino group can be removed to yield dedimethylamino compounds, such as 11, by the action of zinc in aqueous acetic acid. Dedimethylamino-12a-deoxytetracyclines, such as 12, being the by-products, can be obtained as major products by simply increasing the reaction time[9,36]. Methods[37,38] are available for the selective removal of the 12a-hydroxy group. One of these[37] calls for the use of zinc dust in

dilute ammonium hydroxide. For example, from tetracycline (3), the 12a-deoxy compound 13 is obtained. The orientation of the dimethylamino group in 13 has not been settled. Since rehydroxylation at C-12a can be achieved in the right stereochemical sense, the normal active compound with the α-configuration can be regenerated[39]. However, in one case[40] the inactive epi compound was obtained, suggesting a β-orientation for the C-4 dimethylamino group in 13.



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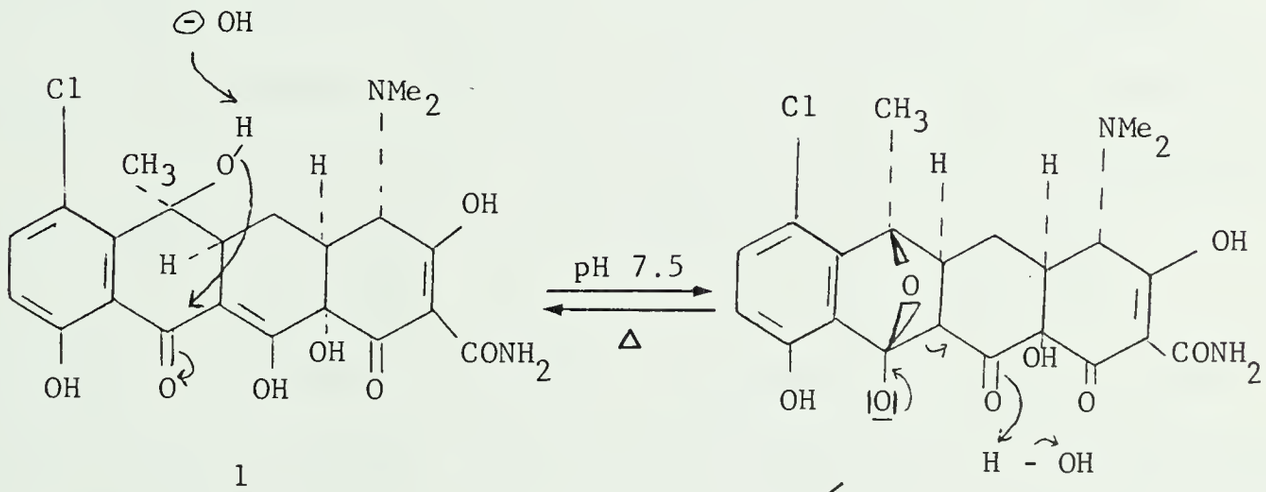

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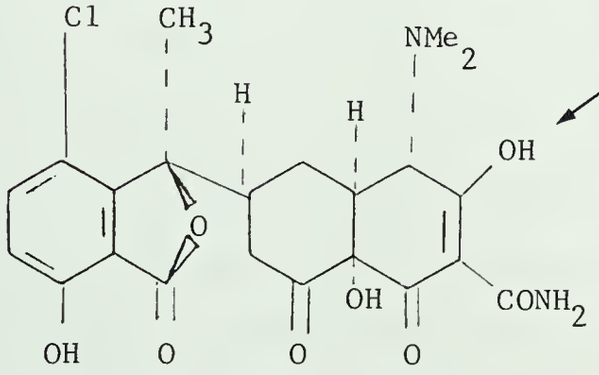

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4. Syntheses and General Pharmacological Activity of Tetracycline Analogs

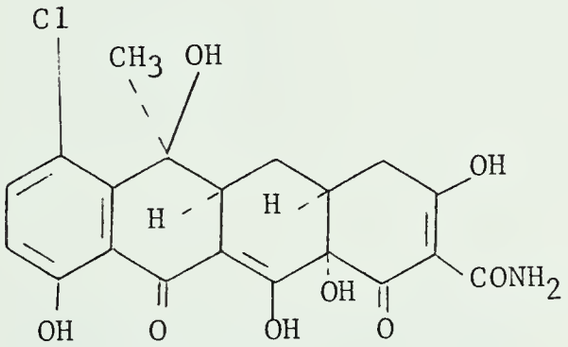
Many new tetracyclines with antibacterial properties have been developed *via* chemical modifications of readily available tetracyclines, namely tetracycline (3), oxytetracycline (2), 6-demethyltetracycline, and 6-demethylchlortetracycline (4). The two last-mentioned tetracyclines are produced by a mutant Streptomyces aureofaciens. The acid-stable 6-deoxytetracyclines enable modifications to be made at positions 7 and 9 by electrophilic substitution reactions. Products of modifications are discussed with respect to their syntheses and pharmacological activities. *In vitro* and *in vivo* activities are specified, whenever possible.

Modifications at Position 2

Pyrrolidinomethyltetracycline (rolitetracycline 5) typifies one of the many C-2 modified tetracyclines derived from the Mannich reaction. It is prepared by reacting tetracycline (1) with formaldehyde and pyrrolidine[41,42]. In view of the ease of regeneration of the parent tetracycline compound in aqueous solutions, its tetracycline-like antibacterial activity is not surprising[43]. Furthermore, when used parenterally, pharmacodynamic advantages are noted[44]. Treatment with *p*-toluenesulfonyl chloride in pyridine smoothly converts the tetracyclines to the corresponding nitrile derivatives[45] (e.g. 14, 15) which do not show any significant activity. Fermentation derived 2-acetyl-2-decarboxamido-tetracycline (16) and 2-acetyl-2-decarboxamido-chlortetracycline (17) possess ten to thirty per cent tetracycline activity[46]. Replacement of one of the carboxamide hydrogens with a methyl group does not affect the *in vitro* antibacterial activity[47] of the tetracyclines. Large alkyl groups have an adverse effect on activity through a combined interplay of structural

effect and highly lipophilic character of the compound[8]. For example, N-t-butyl-6-demethyl-6-deoxytetracycline (18) shows moderate *in vitro* activity against gram-positive bacteria but has lost *in vivo* activity. Compound 18 was prepared from the corresponding nitrile with isobutylene under strongly acidic conditions[48].

The 2-carboxamido group has also been replaced by an aldehyde[49] and an aldimine[50] with substantial loss of antibacterial activity in both instances.



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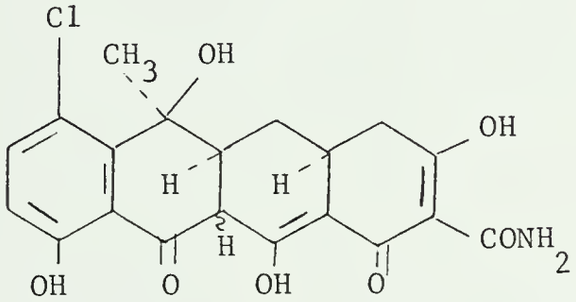

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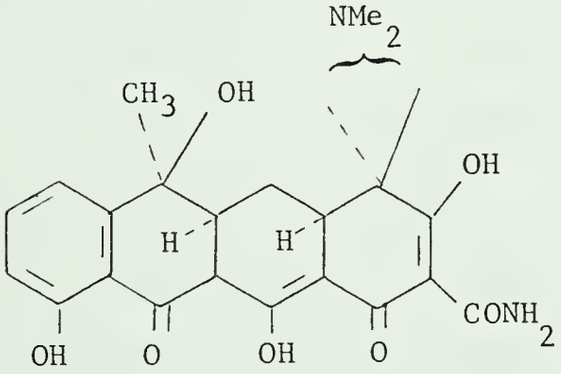

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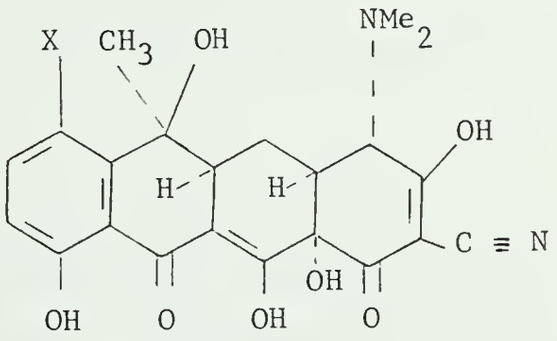
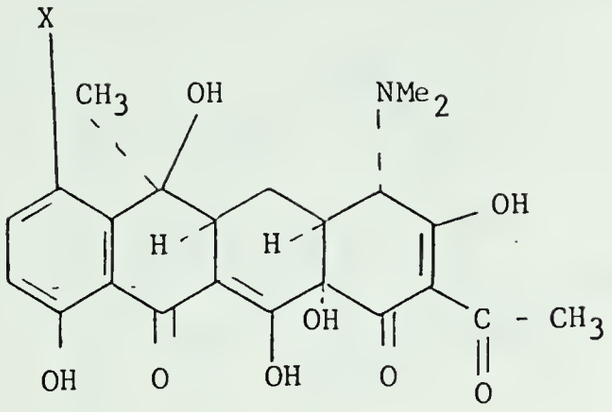


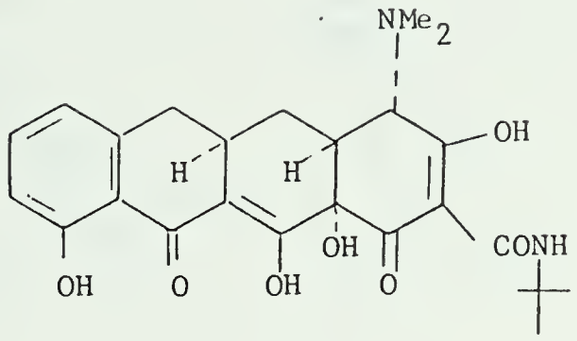
14 x = H

15 x = Cl



16 X = H

17 X = Cl



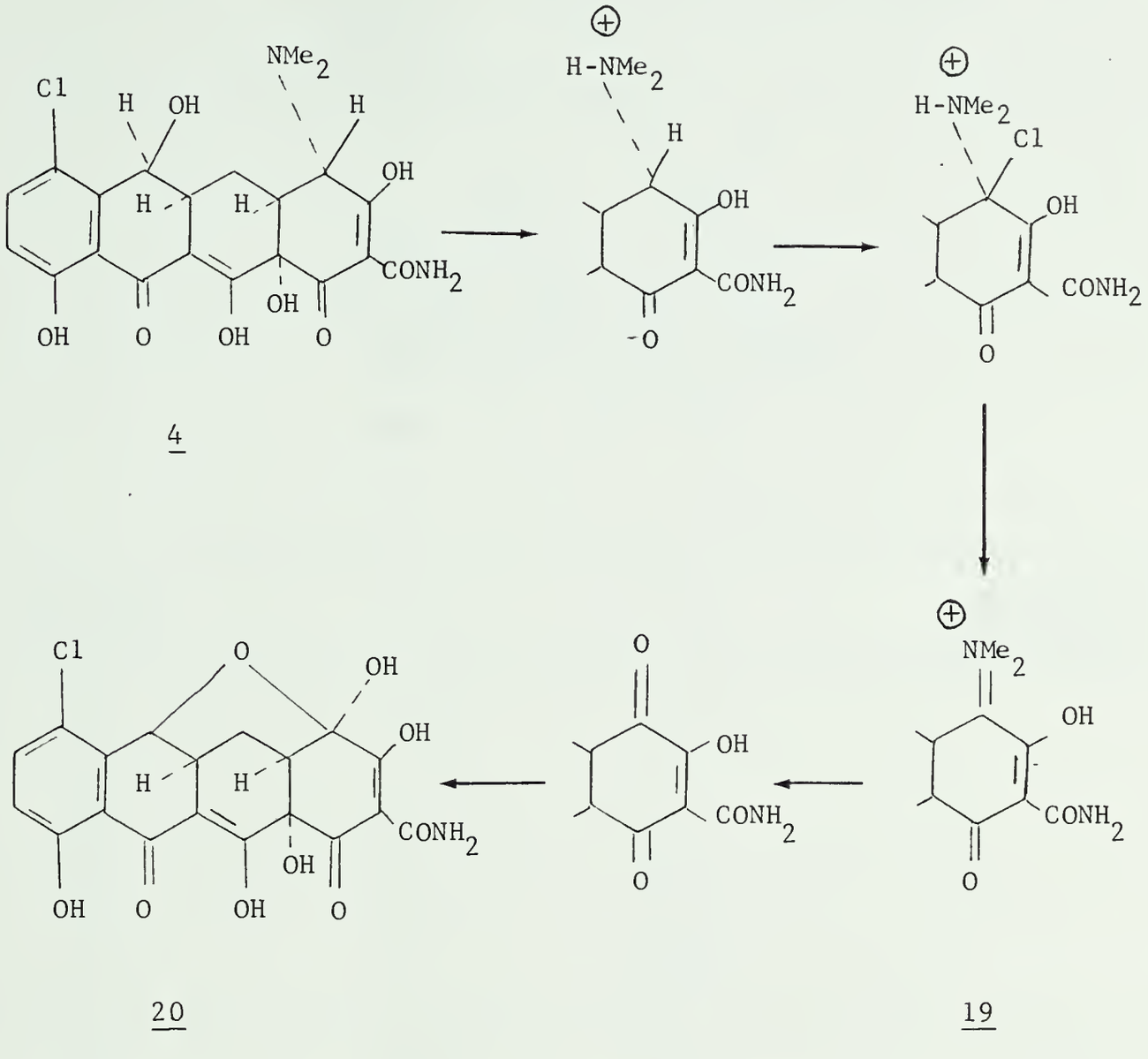
18

Modifications at Position 4

The basic amine function at C-4 is required for useful *in vivo* activity since known dedimethylamino compounds afford no significant *in vivo* activity, particularly against gram-positive bacteria. The α-configuration of the C-4 dimethylamino group is essential for high antibiotic activity; 4-epi tetracyclines have only weak inhibiting action *in vitro* and none at all *in vivo*[8].

Under appropriate conditions, sources of positive halogen (e.g. N-chlorosuccinimide) as well as a variety of other reagents in acidic media can promote tetracycloxide formation.[51,52] The reaction is believed to involve the formation of a ternary iminium compound 19 which would be subject to attack by water to give the 4-keto analog, which in turn undergoes hemiketal formation with the C-6 hydroxyl to give the 4-hydroxytetracycloxide, exemplified by structure 20 (scheme 1).

Scheme 1: Mechanism of Tetracycloxide Formation



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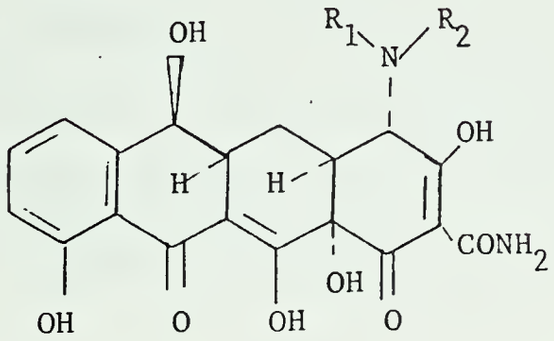
19

20

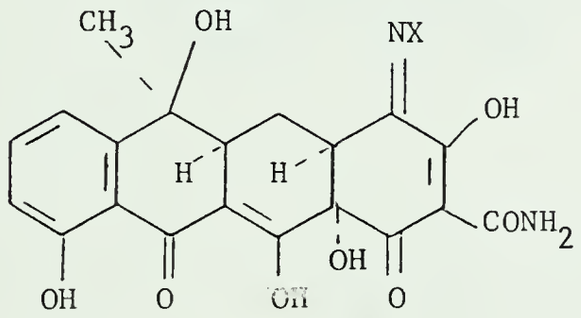
Table 2: C-4 Modified Tetracyclines[52]



Compound	R_1	R_2	In Vitro activity (tetracycline = 100)
21	H	H	
22	H	CH_3	
23	H	C_2H_5	
24	H	$n\text{-}C_3H_7$	
25	H	C_2H_4OH	
26	CH_3	CH_3	96
27	CH_3	C_2H_5	75
28	CH_3	C_3H_7	50
29	C_2H_5	C_2H_5	25
30	CH_3	C_2H_4OH	

4-Hydroxytetracycloxide has been reductively aminated[52], catalytically, with simple aliphatic primary amines to give the 4-dedimethylamino-4-alkyl-amino-6-demethyltetracyclines 21 to 25. These products were alkylated with simple aldehydes to yield the 6-demethyltetracyclines 26 to 30. The 4-dialkylamino analogs have depressed activity relative to the parent dimethylamino compound. The decrease in activity is correlated with the bulkiness of the dialkylamino function, as can be seen from Table 2.

The 4-hydroxytetracycloxide 20, the oxime 31 and hydrazone 32, derived from it by treatment with hydroxylamine and hydrazine respectively, show little or no antibacterial activity.[14]

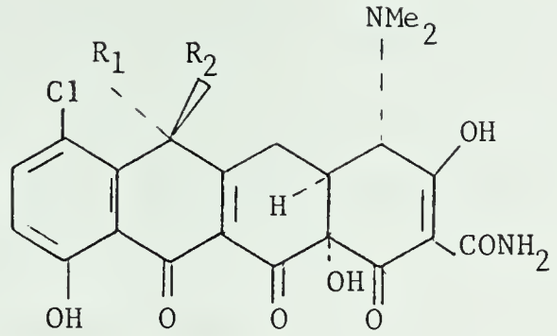


31 X = OH

32 X = NH_2

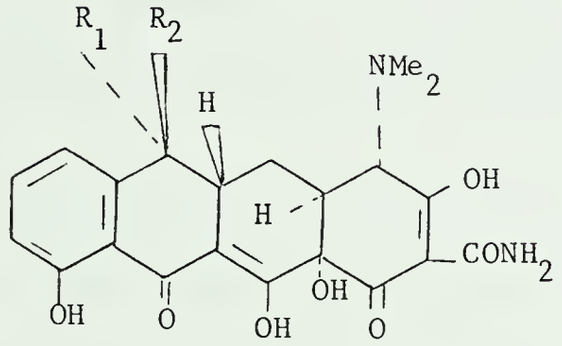
Modifications at Position 5a

5a(11a)-Dehydrochlortetracycline 33 with the usual configuration at C-6 is inactive against Staphylococcus aureus, whereas 5a(11a)-dehydro-6-epichlortetracycline 34 is quite active[53]. Again, 5a-epitetracycline 35 is inactive, whereas 5a-epi-6-epi-tetracycline 36 is active. A meaningful correlation of these results is not immediately evident. Nonetheless, they may indicate that the C-5a asymmetric center is not necessarily a critical feature for activity.



33 $R_1 = CH_3$, $R_2 = OH$

34 $R_1 = OH$, $R_2 = CH_3$



35 $R_1 = CH_3$, $R_2 = OH$

36 $R_1 = OH$, $R_2 = CH_3$

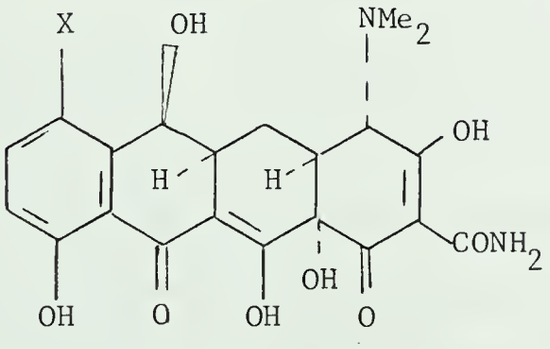
Modifications at Position 6

The C-6 benzylic hydroxy function in the molecule of the fermentation derived tetracyclines presented a center of instability which for many years limited the scope of chemical reactions applicable to the tetracycline antibiotics. Independent studies by McCormick[54] and Stephens[55] led to the development of acid stable derivatives involving removal of the hydroxy group at C-6 by catalytic hydrogenolysis. This important discovery not only yielded many C-6 modified tetracycline derivatives

of high antibacterial activity but also has made possible substitution of the aromatic ring D, a process which normally requires the presence of strong acid. The two fermentation-derived 6-demethyltetracyclines 4 and 37 are equivalent to tetracycline (1) in activity[13].

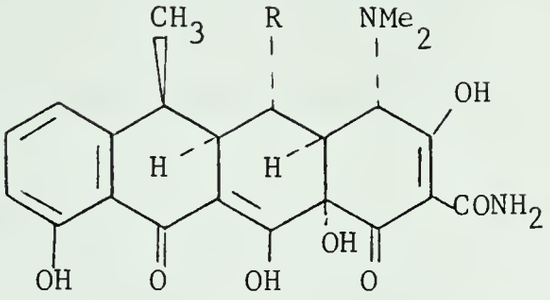
Catalytic hydrogenolysis (with stereochemical inversion at C-6)[48] of fermentation tetracyclines (3) and (2) afforded β-6-deoxytetracyclines 38 and 39 both of which have high activity. α-6-Deoxytetracyclines (with natural configuration at C-6), available from the corresponding 6-methylenetetracyclines such as 6, are generally more active than the β-epimers.

Hydrogenation of 4 leads to 6-demethyl-6-deoxytetracycline (40). This compound is of particular interest since it is the simplest known tetracycline with broad-spectrum activity, both *in vitro* and *in vivo*. This compound shows that neither the 6-methyl nor the 6-hydroxy group is essential for antibacterial activity. From a stereochemical viewpoint, 40 may represent the minimal structural asymmetry in a compound necessary for conferring useful tetracycline antibacterial activity[48].



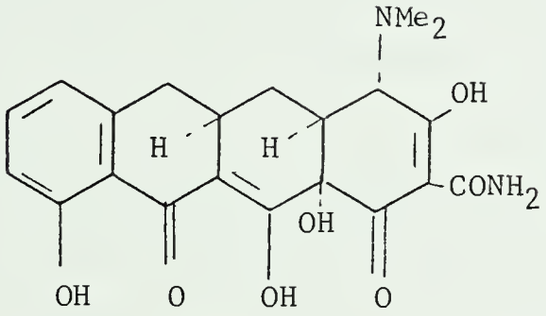
4 X = Cl

37 X = H



38 R = H

39 R = OH



40

Methacycline (6) is another important C-6 modified derivative with excellent activity. Its synthesis by Blackwood et al.[56] begins with a reaction of oxytetracycline (2) and N-chlorosuccinimide in 1,2-dimethyloxyethane. The product, 11a-chlorotetracycline 6,12-hemiketal (41), undergoes exocyclic dehydration with anhydrous hydrogen fluoride to yield the 6-methylene derivative 42 from which the halogen is subsequently removed by sodium hydrosulfite in water. (Scheme 2).

6-Methylenetetracyclines have been used as intermediates in the preparation of sulfur-containing tetracyclines[57] such as 43. These compounds are useful in lipophilicity studies on in vitro activity

The phenyl and benzyl mercaptan adducts are highly lipophilic and show markedly depressed activities. However, considerable activity is regained[8] by converting them to their more polar sulfoxides 44.

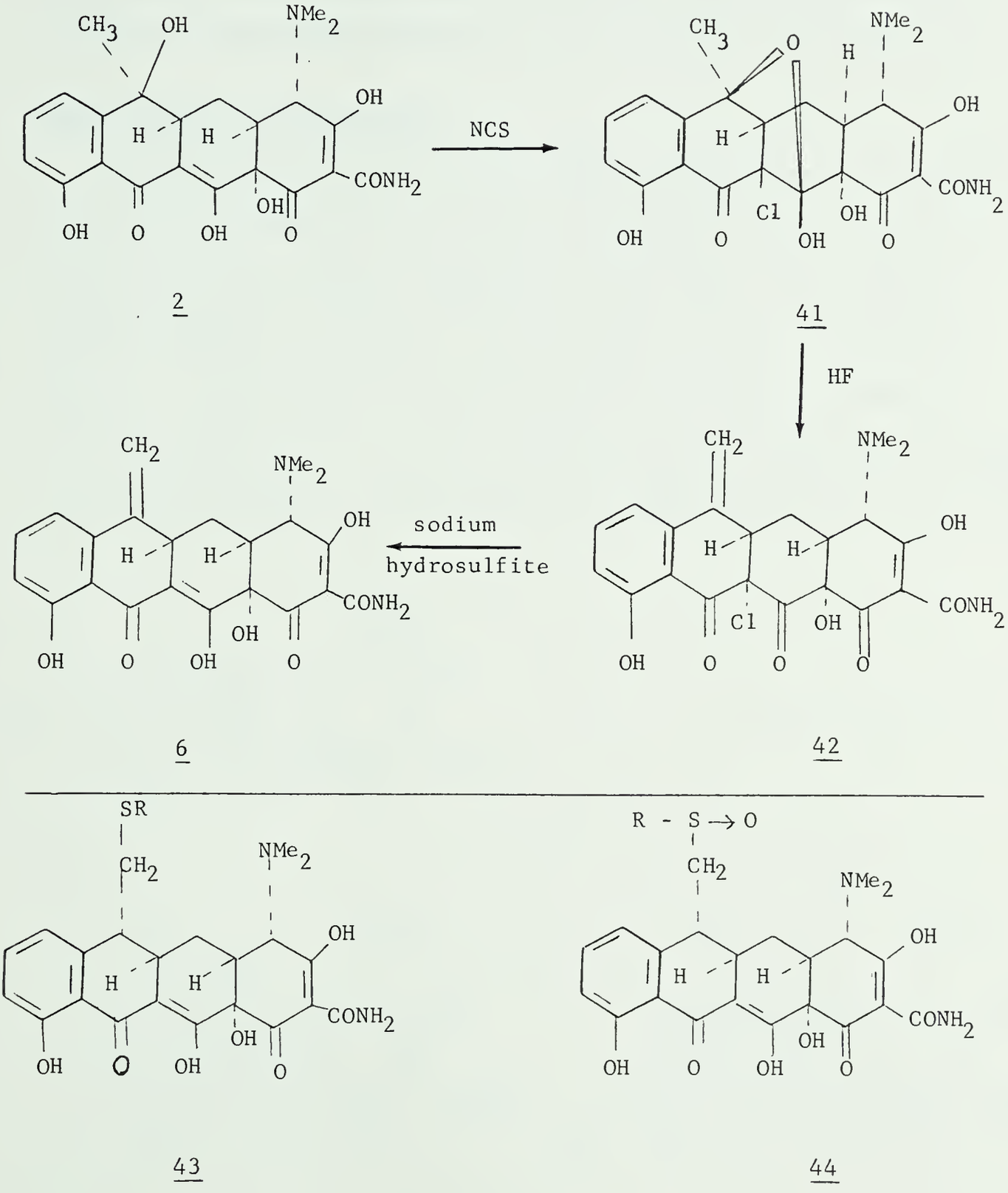
Modification at Positions 7 and 9

Electrophilic substitution of the acid-stable 6-deoxytetracyclines yields an extensive series of C-7 and/or C-9 substituted analogs. Substitution at the C-7 position is more advantageous from the standpoint of activity than at C-9, presumably because of steric effects and/or H-bonding of the latter substituent with the phenolic hydroxy at C-10 disrupting an active site in the tetracycline molecule. Electron withdrawing substituents such as nitro, chloro and bromo groups at the C-7 position clearly enhance *in vitro* activity since these derivatives are invariably more active than the corresponding unsubstituted compounds. The amino function can be either strongly electron withdrawing or donating depending upon whether or not the amine is protonated. This is in accord with the fact that 7-amino-6-demethyl-6-deoxytetracycline is less active *in vitro*[8] than the stronger base 7-dimethylamino-6-demethyl-6-deoxytetracycline (minocycline[8]) which is more apt to be protonated[8]. Minocycline (8), which is the newest member of the tetracycline antibiotics, possesses enhanced activity towards a series of gram-positive staphylococcal strains that are already resistant to other tetracyclines.

Modifications at Position 11a

11a-Halogenated tetracyclines, for example 45, are the most common analogs in this class. Substituents at this position block the extended conjugation of the keto-enol system from C-10 to C-12, which is essential

Scheme 2: Synthesis of 6-Methylenetetracycline



2 41 42 6 43 44

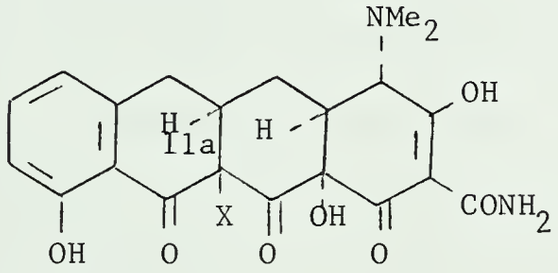
R = phenyl or benzyl

for antibacterial activity[48]. Thus 11a substituted tetracyclines show diminished *in vitro* activities.[58] However, considerable *in vivo* activity was noted for those substituted tetracyclines which can regenerate the parent compound by elimination of the substituent in question.

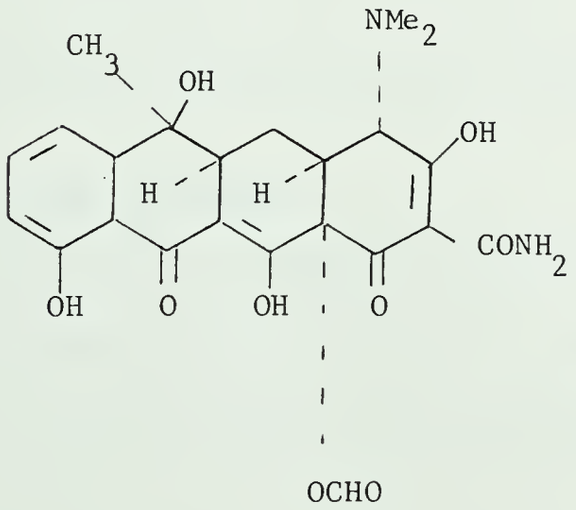
Modifications at Position 12a

O-12a-Formyltetracycline 46 is formed by treating a cold pyridine solution of tetracycline (3) with excess acetoformic acid reagent (a solution of acetic-formic anhydride in acetic acid). The compound rapidly undergoes solvolysis to tetracycline in basic or in neutral solution. At pH = 2, the half-life for the hydrolysis to tetracycline at 25° is six hours[38]. Thus the compound possesses equivalent *in vitro* and in vivo activity to tetracycline. All higher esters are less active[59].

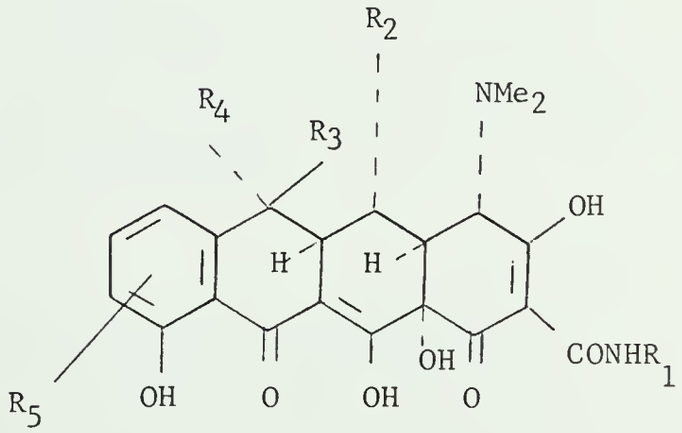
45



X = Cl, Br, or F



46



47

Summary of Structure-Activity Relationships

The basic structural features, namely the configuration of the asymmetric centers at C-4, C-4a and C-12a are essential for antibacterial activity. One of the carboxamide hydrogens may be replaced by a methyl group or other substituent which can be hydrolyzed in water to regenerate the parent tetracycline. The basic amino function at C-4 is not needed for *in vitro* activity, but its presence, and in the correct α-configuration is essential for *in vivo* activity. Furthermore, the dimethylamino group may be substituted by a primary amino group, but larger alkyl substituents have an unfavorable effect on activity. The functional groups from C-5 to C-9 may be altered or removed to arrive at compounds with increased chemical stability and antibacterial activity.

The two extended conjugated chromophoric systems of the A-ring and BCD-ring may not be altered. Attempts to do so by opening one of the rings (e.g. 10) or by blocking of one of the chromophores (e.g. 45) leads to destruction of activity. Therefore, characteristic chemotherapeutic properties of the tetracycline antibiotics are dependent upon the maintenance of all of the structural, stereochemical and chromophoric features, as illustrated in 47, wherein the groups R_1 to R_5 are the only functions which may be varied by certain substituents without inflicting a substantial loss in antibiotic activity.

5. Total Syntheses

The complex arrangement of functional groups and stereochemistry of the tetracyclines has inspired a number of research teams to attempt their total syntheses. The highlight of this effort culminated in the

successful synthesis of the fully biologically active 6-demethyl-6-deoxytetracycline (40 and its enantiomer).

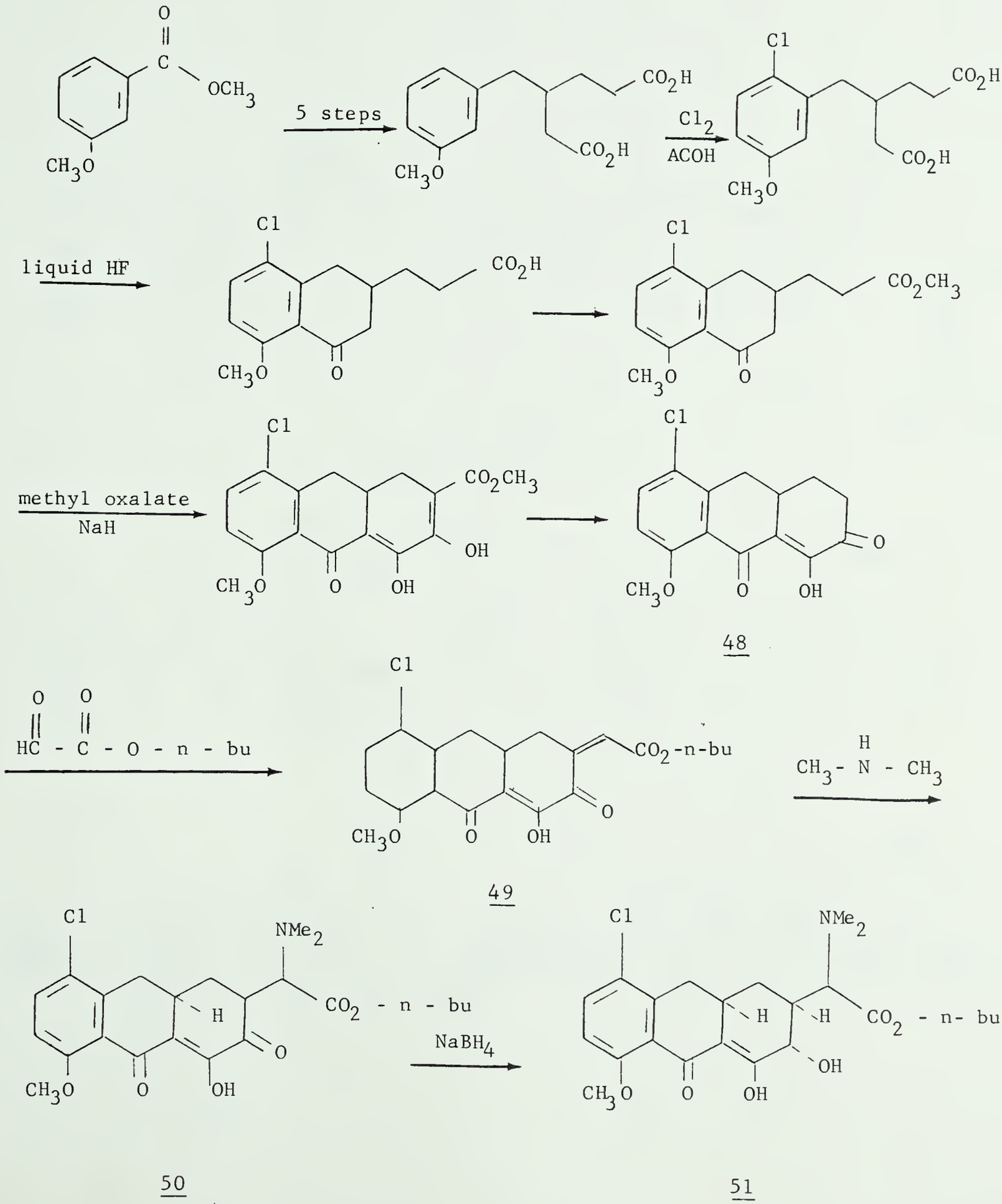
It was first synthesized by Woodward et al.[60] in 1962. Details of that synthesis were published[61] in 1968. It was immediately apparent that the construction of ring A of the molecule would be the most formidable task, since every carbon atom of the skeleton of that ring bears a substituent and three of the four asymmetric centers of the molecule are situated on C-4, C-4a and C-12a of the ring A. The strategy employed was to begin with the aromatic ring D, onto which ring C and B were to be built in a stepwise fashion by a series of condensation reactions (Scheme 3). The key intermediate, therefore, is the compound 48 onto which the ring A is to be attached via the α-carbon of the terminal carbonyl by condensation with n-butyl-glyoxalate to afford 49. Dimethylamine then adds stereospecifically to the exocyclic double bond, giving 50. The terminal carbonyl is reduced with sodium borohydride yielding 51 which lactonizes in refluxing toluene and p-toluenesulfonic acid. The lactone 52 is reduced by zinc dust in formic acid to yield the acid 53. After catalytic dehalogenation the acid is converted into a mixed anhydride 54. Ethyl-N-(tert-butyl) malonamate 55 plays a key role as a convenient means of introducing the carboxamide function of an eventual tetracycline in a protected form from which the protective group could be readily released. Accordingly, the magnesium salt of ethyl N-(tert-butyl) malonamate is acylated with 54 to give 56. Cyclization of the latter compound by use of sodium hydride as a condensation agent gives 57. Smooth cleavage of both the N-tert-butyl and the O-methyl groups is effected with hydrobromic acid giving 58. Stereospecific introduction of the hydroxy group at C-12a by autoxidation is

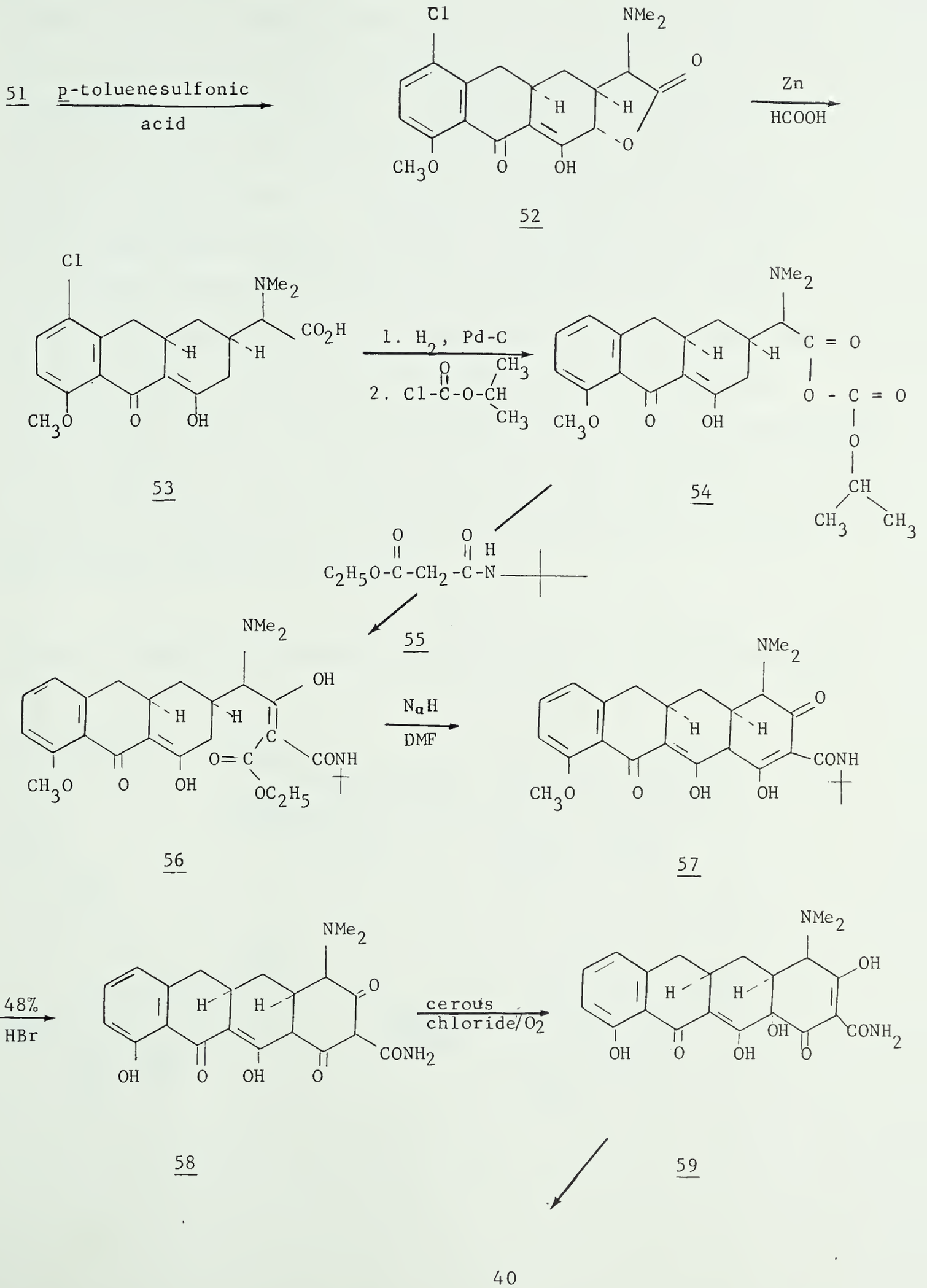
achieved by carefully controlled oxygenation of racemic 58 in the presence of cerous chloride in buffered methanol-DMF solution, giving 59 in 6.5% yield[39]. Separation and purification of 59 and the inversion of the 4-dimethylamino group to the natural α-configuration were the other difficult steps which had to be overcome before pure crystalline totally synthetic 6-demethyl-6-deoxytetracycline (40 and its racemate) was obtained. The synthetic compound is only half as active as its optically active counterpart, indicating that one optical isomer is completely devoid of biological activity.

In 1965 Muxfeldt and Rogalski obtained the same racemic 6-demethyl-6-deoxytetracycline by a different but simpler total synthesis which for the first time allowed tetracyclic compounds to be prepared on a large scale[62]. Applying the main scheme of the synthesis, the most complicated and chemically more active of the natural tetracyclines, oxytetracycline (2), was successfully synthesized in 1968[63]. For purposes of comparison and contrast with Woodward's synthesis, the Muxfeldt method is illustrated for the preparation of 6-demethyl-6-deoxytetracycline (Scheme 4). The use of the 7-Cl blocking group to control direction of cyclization and the introduction of the 12a-OH by autoxidation are the common features in both syntheses.

The tetralone acid 61 is made from 1-chloro-2-bromomethyl-4-methoxybenzene (60) by a condensation reaction. 61 is transformed to the aldehyde 62 in the usual manner[64,65]. Condensation of the aldehyde 62 with hippuric acid yields 63. This reaction is essentially that of the classical Erlenmeyer azlactone synthesis[66]. Careful deketalization of 63 results in the formation of 64, with two equivalents of sodium hydride, to afford 66, by double ring closure in which three new C-C bonds are created in one step. Although there are three asymmetric

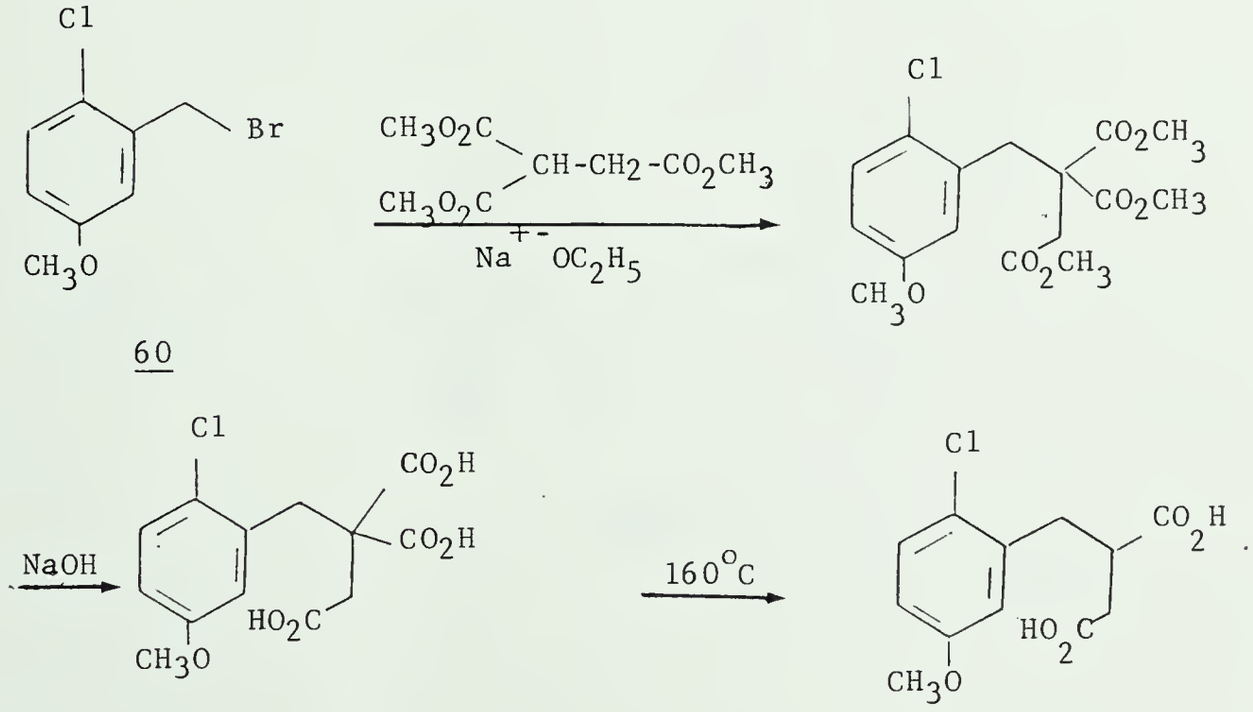
Scheme 3: Synthesis of 6-demethyl-6-deoxytetracycline (40) by Woodward et al.[60]

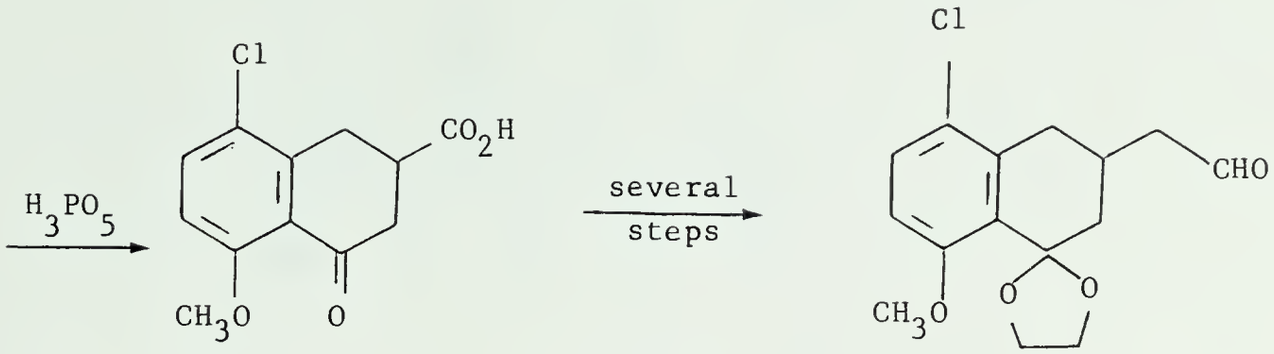



51
p-toluenesulfonic acid
Cl
NMe2
O
H
H
O
CH3O
O
OH
52
Zn
HCOOH
Cl
NMe2
CO2H
H
H
CH3O
O
OH
53
1. H2, Pd-C
2. Cl-C-O-CH
O
CH3
CH3
NMe2
C = O
O - C = O
O
CH
CH3
CH3
54
O
O
H
C2H5O-C-CH2-C-N
55
NMe2
OH
H
H
C
O=C
CONH
CH3O
O
OH
OC2H5
56
NaH
DMF
NMe2
O
H
H
CONH
CH3O
O
OH
OH
57
48%
HBr
NMe2
O
H
H
CONH2
OH
O
OH
O
58
cerous chloride/O2
NMe2
OH
H
H
OH
CONH2
OH
O
OH
O
59
40

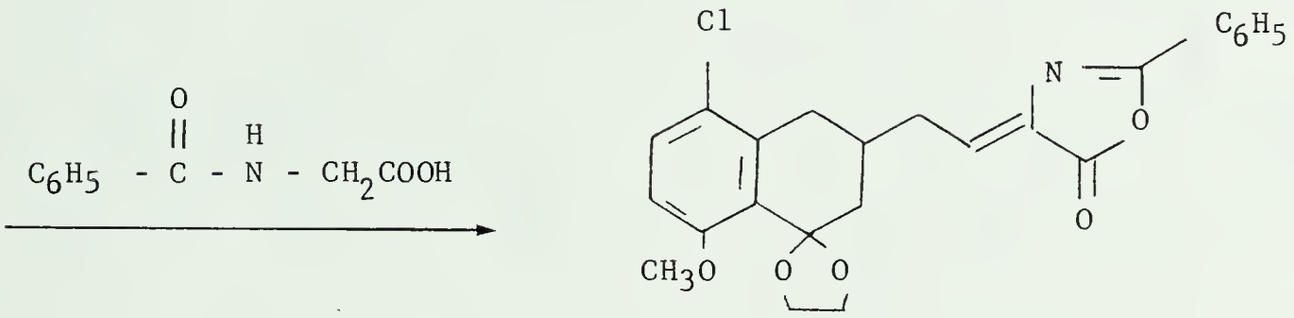
centers in 66, four compounds (not eight) are to be expected, 67 to 70. This is because it was known from previous experiments[67] that the C-4a hydrogen occupied only the α-configuration in this reaction. These four compounds were separated by chromatography followed by fractional crystallization. It should be noted that the C-4 epimer 68 can be epimerized in pyridine to give 67. The N-benzoyl group in 67 is removed by Meerwein's reagent[68] to yield 71. Both the N-t-butyl group and the O-methoxy group are removed in one step by treatment with hydrobromic acid. Alkylation of the nitrogen at C-4 is achieved by hydrogenation in the presence of triethylamine and an excess of formaldehyde with palladium-on-charcoal catalyst, giving 73. Stereospecific introduction of the C-12a hydroxy group is achieved in satisfactory yield by autoxidation with molecular oxygen and platinum[62].

Scheme 4: Synthesis of 6-demethyl-6-deoxytetracycline by Muxfeldt et al.[63]

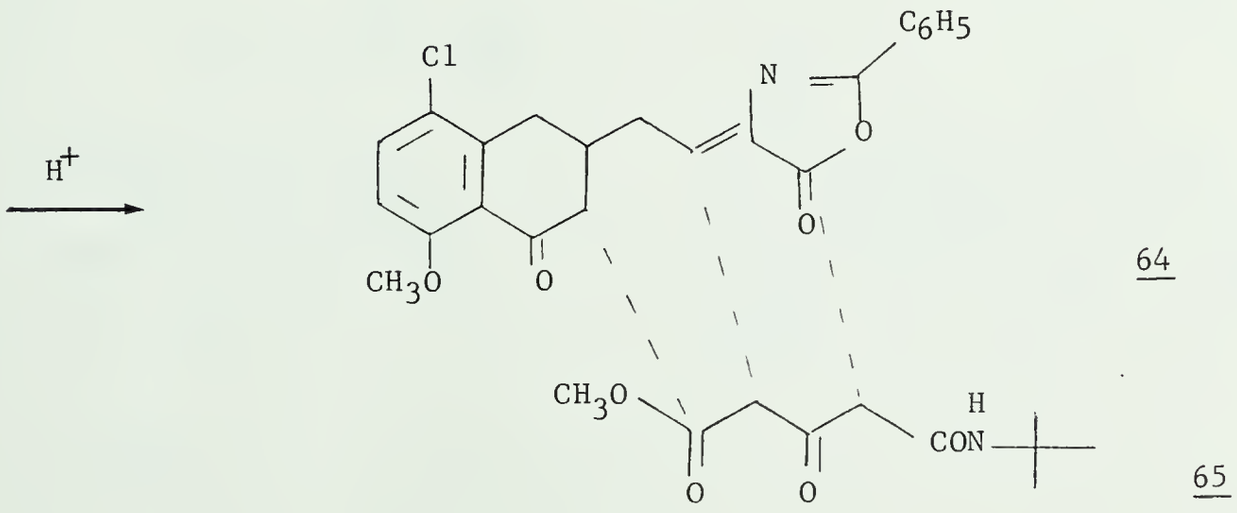



H_3PO_5
Cl
CO_2H
CH_3O
O
several
steps
Cl
CHO
CH_3O
O
O

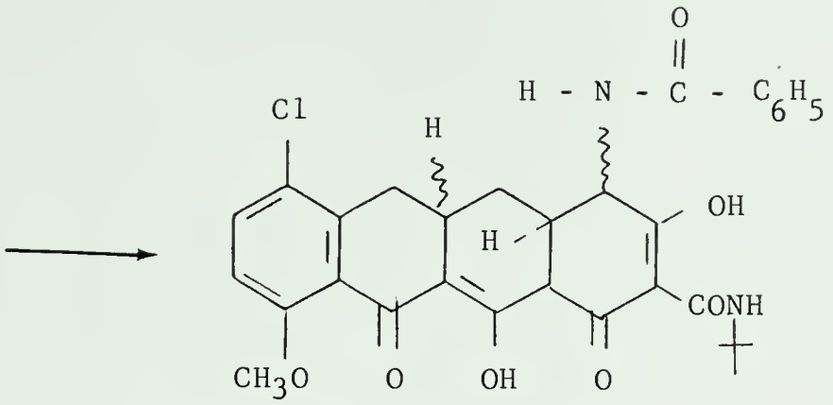
61 62


O
H
C_6H_5 - C - N - CH_2COOH
Cl
C_6H_5
N
O
O
CH_3O
O
O

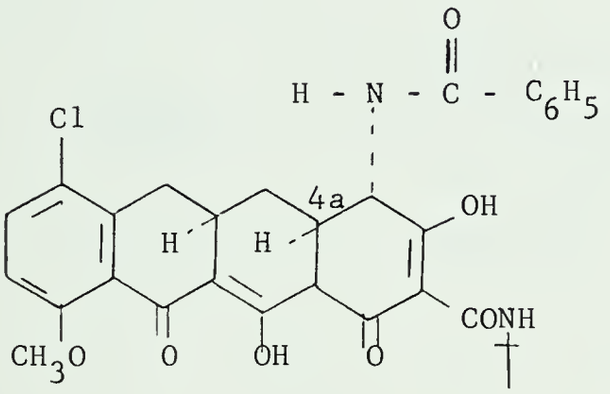
63


H^+
Cl
C_6H_5
N
O
O
CH_3O
O
CH_3O
H
CON
O
O

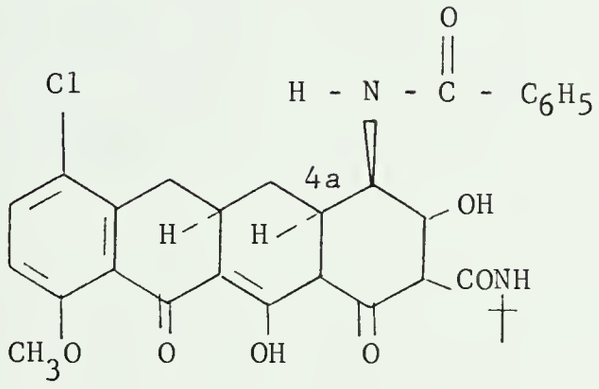
64 65



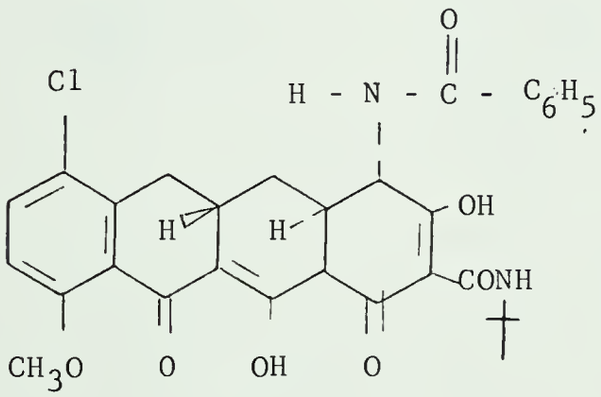
66



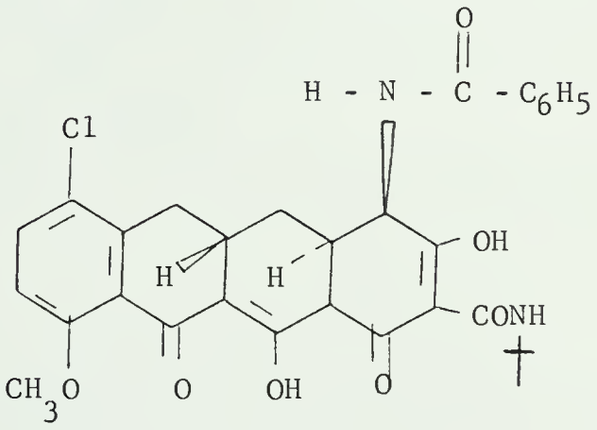
67



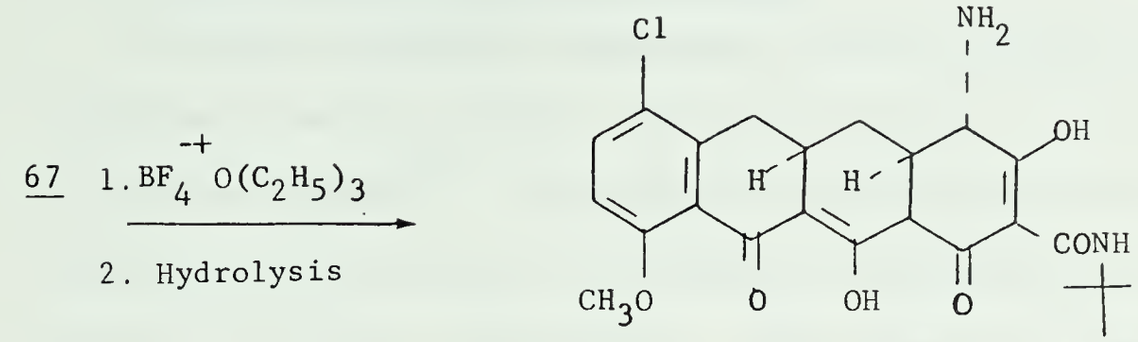
68



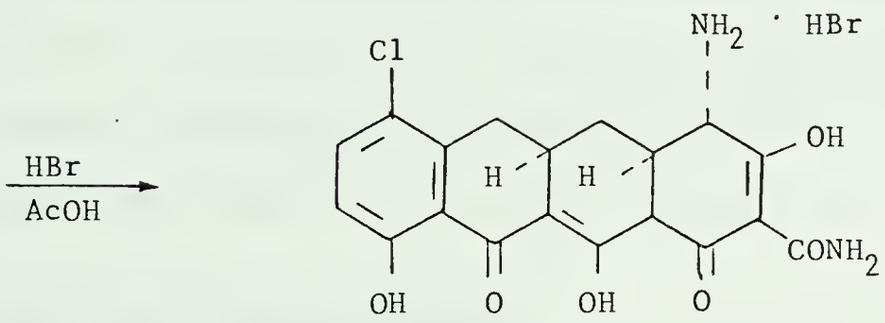
69



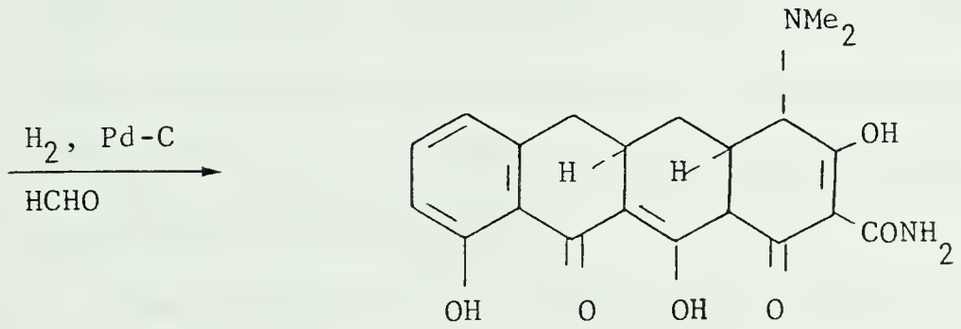
70


67
1. BF_4^- $O(C_2H_5)_3$
2. Hydrolysis
Cl
NH_2
OH
H
H
CONH
CH_3O
O
OH
O

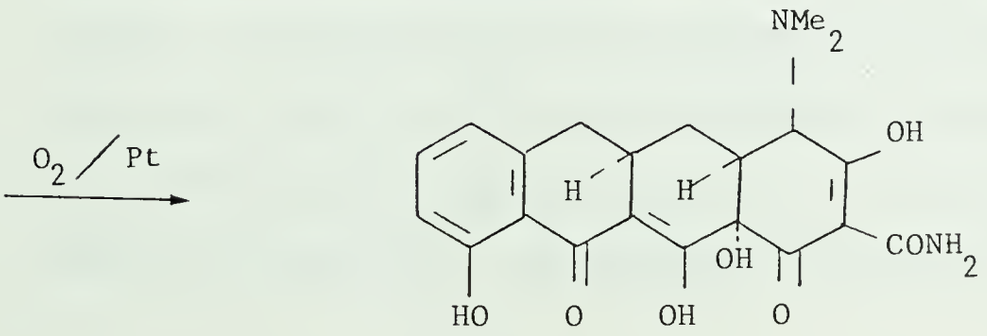
71


HBr
AcOH
Cl
NH_2 · HBr
OH
H
H
$CONH_2$
OH
O
OH
O

72


H_2, Pd-C
HCHO
NMe_2
OH
H
H
$CONH_2$
OH
O
OH
O

73


O_2 / Pt
NMe_2
OH
H
H
$CONH_2$
OH
HO
O
OH
O

40

6. Syntheses of Condensed Ring Analogs

The only tetracycline review which contains a fairly comprehensive reference of smaller synthetic tetracycline analogs remains the one by G.C. Barrett in 1963[13]. The majority of these analogs are model compounds synthesized in the process of preliminary investigations into the total synthesis of natural tetracyclines. Some attempts were made on the syntheses of tetracycline analogs having the extended conjugated system from C-10 to C-12 in tetracyclines. This system constitutes one of the possible sites in tetracyclines for chelation with metal ions[58]. However, no significant activities have been observed for any of these compounds.

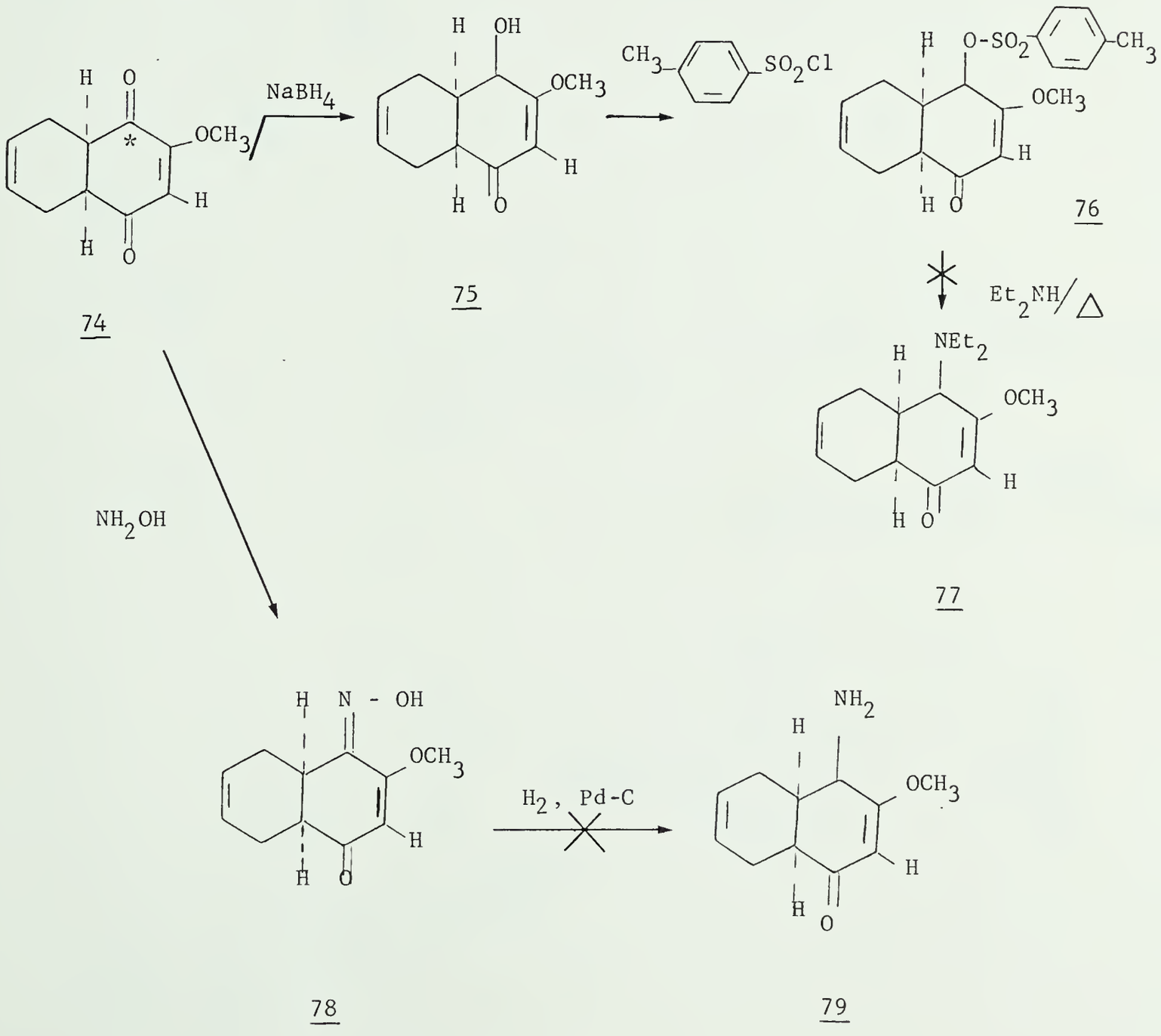
Syntheses of Ring A Analogs

In contrast, much more specific effort has gone into the syntheses of ring A analogs. The A-ring possesses a basic site at C-4 and an acidic site due to the presence of the unique tricarbonyl methane system. These structural features naturally drew considerable interest from the standpoint of their possible correlation with the remarkable antibacterial activities of tetracyclines.

The synthesis of a two-ring model compound 77 (or 79) was attempted[69] which shared the cis ring fusion as well as a general structural similarity with ring A of the tetracyclines. Selective reactions at the starred carbonyl of 2-methoxy-4a,5,8,8a-tetrahydronaphthoquinone (74) were first effected by reduction with sodium borohydride to the keto-alcohol 75. Its p-toluenesulfonate 76 was prepared but no reaction took place in refluxing diethylamine. The monoxime of 74 was also prepared, i.e.

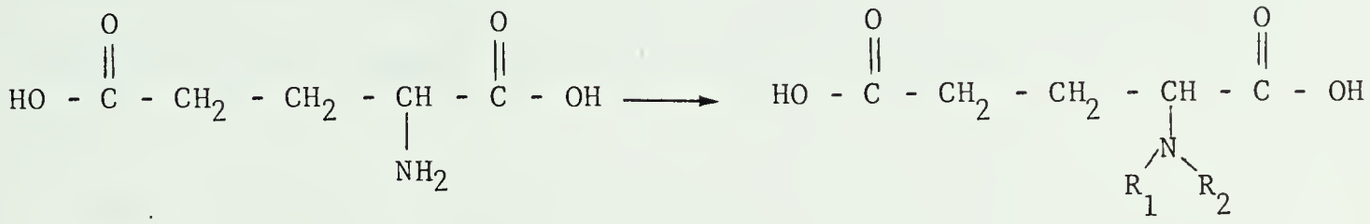
compound 78 by reaction with hydroxylamine, but catalytic hydrogenation with palladium-on-charcoal failed to give the amino compound 79.

Scheme 5: Attempted Synthesis of a Two-ring Ring A Analog

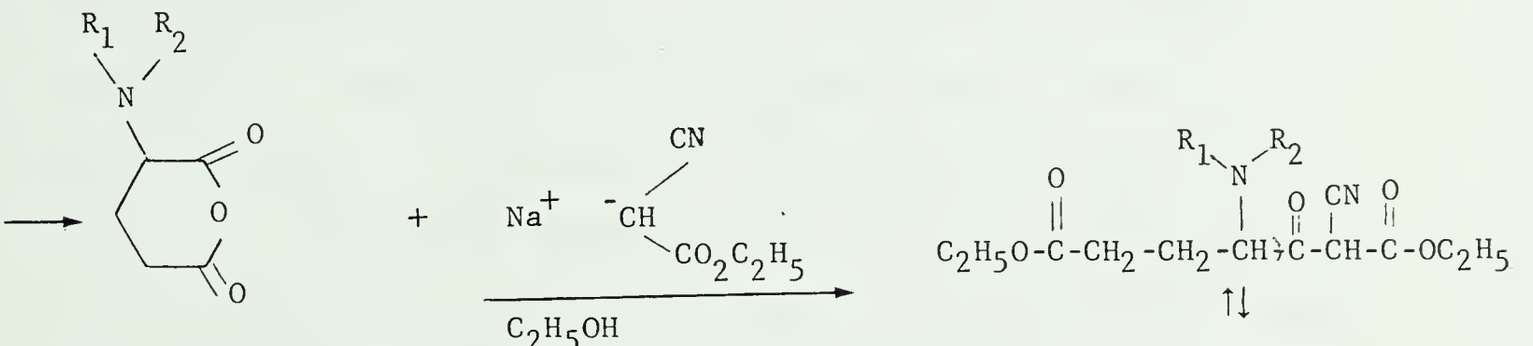


Smissman et al.[70] conceived a convenient way for the preparation of a properly constituted alicyclic molecule 83, which they had hoped would cyclize to give ring A analogs of the type 84.

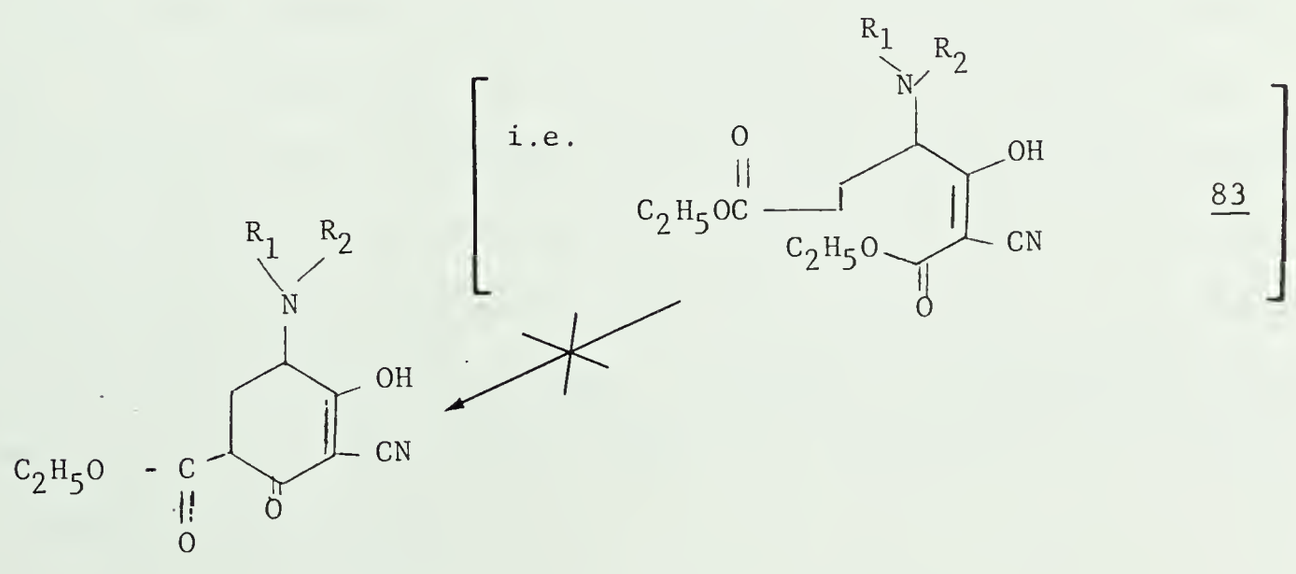
Scheme 6: Attempted Synthesis of a Ring A Analog



80 81



82

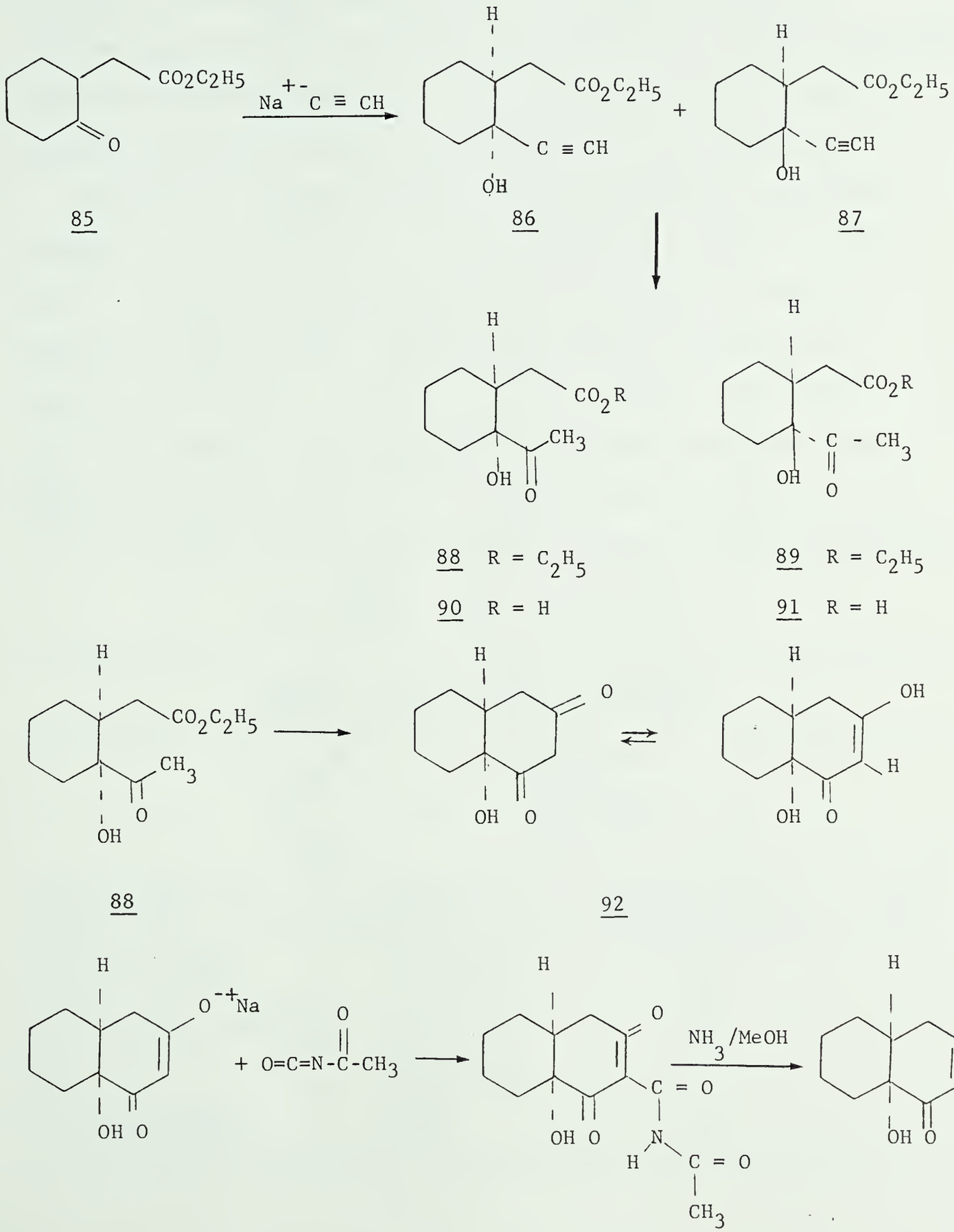


84

Starting from glutamic acid (80), various N-carbethoxyglutamic acids (81) were prepared which were subsequently cyclized to the corresponding anhydrides (82). On treatment of 82 with ethyl sodiocyanoacetate in ethanol the diesters (83) could be obtained. Attempts to cyclize 83 using sodium ethoxide and potassium tert-butoxide failed to give the desired ring A analog 84. Apparently the reaction of 83 with base did not produce the anion at the appropriate site to form 84.

The preparation of cis-2-carboxamidodecalol-9-dione-1,3 (95) was successfully carried out by Shemyakin[71] as one of the many model compounds that he made during the course of studies toward the total synthesis of tetracyclines. Ethynylation of cyclohexanone-2-acetic ester 85 with sodium acetylide in liquid ammonia yielded 86 and its epimer 87 in a 3:1 ratio. The mixture of cis and trans isomers (86 and 87) was not separated at this stage but was subjected to hydration with mercuric acetate in alcohol, followed by saponification to give a mixture of cis and trans acids, 90 and 91. The cis acid 90 crystallized from alcohol more readily and was separated in this manner. Esterification of 90 gave back 88 which was cyclized to cis-9-hydroxy-1,3-diketo-decalin (92). The β-diketone 92 was then carboxamidated by treatment of its Na-enolate with acetyl isocyanate to give the N-acetylcarboxamido compound 94. An examination of the infrared and ultraviolet spectra of starting material 92 and those of 94 showed that reaction did indeed take place at C-2 and not at the C-1 or C-3 oxygen. Ammonolysis of 94 proceeded readily under the action of 20% methanolic ammonia at room temperature to afford the unsubstituted amide 95.

Scheme 7: Synthesis of Cis-2-Carboxamidodecalol-9-dione 1,3

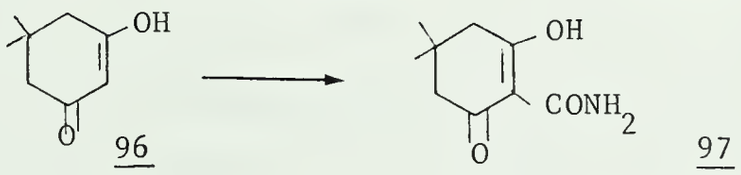


The carboxamidation procedure was first developed by Shemyakin[72]. This method has the merit of giving primarily the C-acylated product in preference to O-acylated side-products. The simple 1,3-diketone, dimedone 96 was chosen in preliminary investigations, and acetyl isocyanate was used as the acylating agent. (Phenylisocyanate was also used, but subsequent ammonolysis of the reaction product was not described).

Since then, a number of modifications of Shemyakin's method have appeared for the construction of the 2-carboxamido 1,3-diketone moiety. Graf[73] synthesized 97 using chlorosulfonyl isocyanate and subsequent hydrolysis in water at 80°. Scarborough and Gould[74] prepared 97 by fusing (or refluxing in a suitable solvent) a mixture of dimedone and urea. The reaction of an aqueous solution of potassium cyanate with dimedone has also been reported[75]. Isocyanic acid is believed to be the reacting species, being generated in situ, from the following equilibrium:

$$KCNO \xrightleftharpoons{H_2O} HCNO \rightleftharpoons HNCO$$

Table 3: Carboxamidation of Dimedone

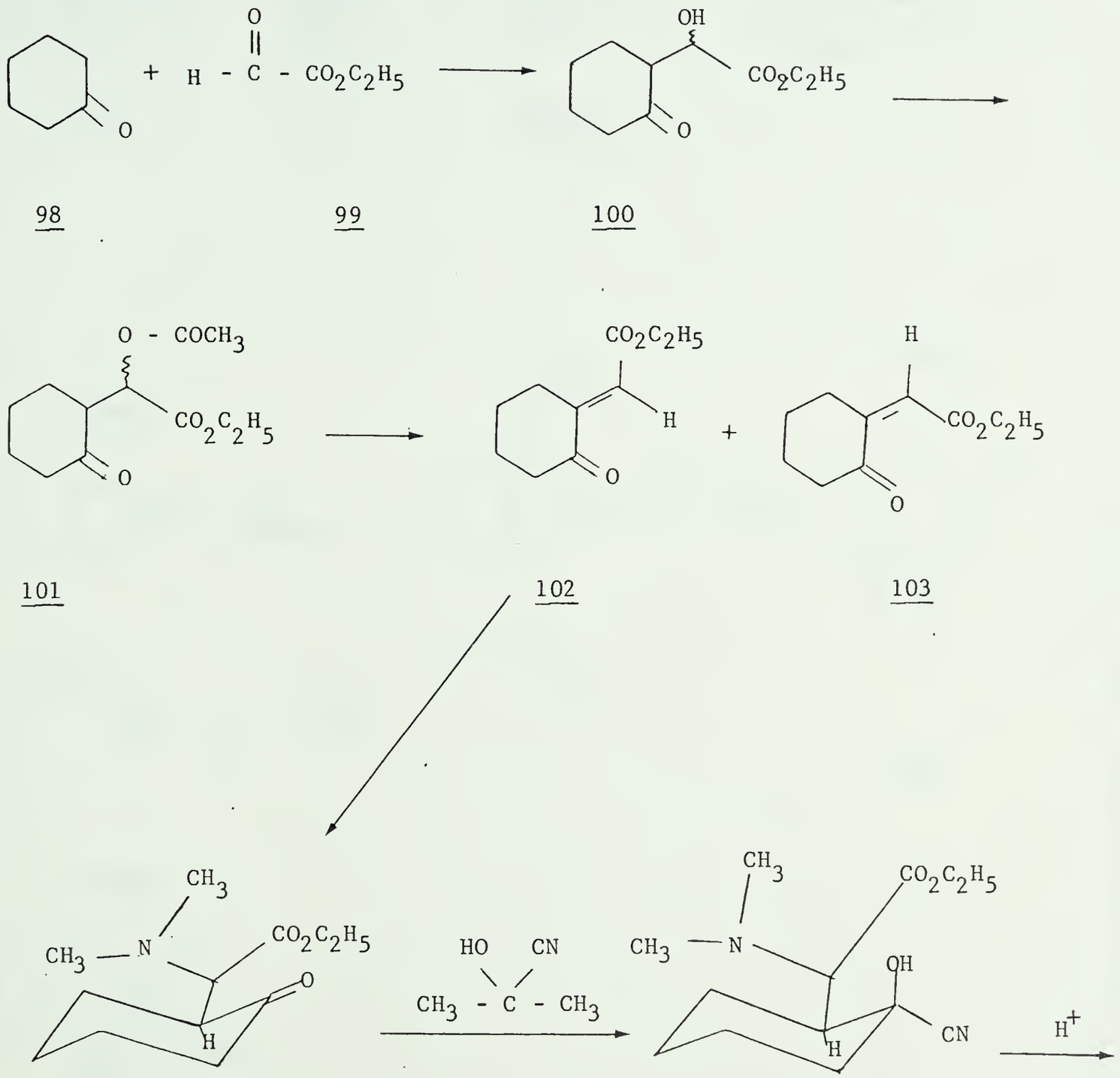


96 97

Reagent used	Formula	Overall yield	Ref
Acetyl isocyanate	$CH_3CO\text{-}NCO$	55%	72
Chlorosufonyl isocyanate	$ClSO_2\text{-}NCO$	49%	73
Urea	$NH_2\text{-}CO\text{-}NH_2$	21%	74
Potassium Cyanate	KCNO	51%	75

Shortly after the synthesis of the 2-carboxamido 1,3-diketone 95, a method for introducing the N,N-dimethylamino group at C-4 of tetracyclines became available[76]. Condensation of cyclohexanone (98) with glyoxyl ester 99 in the presence of pyridine gave rise to a mixture of diastereomeric hydroxy keto-esters (100), which were then subjected to acetylation and desacetoxylation to give the unsaturated keto-ester 102 and its isomer 103. Only the isomer 102 added to dimethylamine to form the ethyl ester of 2-ketocyclohexyl-N,N-dimethylglycine 104. This method of introduction of the N,N-dimethylglycine group into the cyclohexane ring was used by Woodward in his synthesis of 6-demethyl-6-deoxytetracycline (40), described previously. With compound 104 at hand, application of the same series of reactions which led to compound 95, were expected to yield compound 107, which resembles fully the A-ring of tetracyclines. However, Shemyakin et al. were unsuccessful in this approach and reported instead the synthesis of 106[77] (Scheme 8). It is important to note that compound 106 does not have a cis ring fusion as in the pharmacologically active tetracyclines. This came about because of the stereospecific addition of HCN to the carbonyl in 104. This was to be expected since the dimethylglycine residue would impose a steric and/or electronic effect on the incoming cyanate anion. Carboxamidation of compound 106 was not reported.

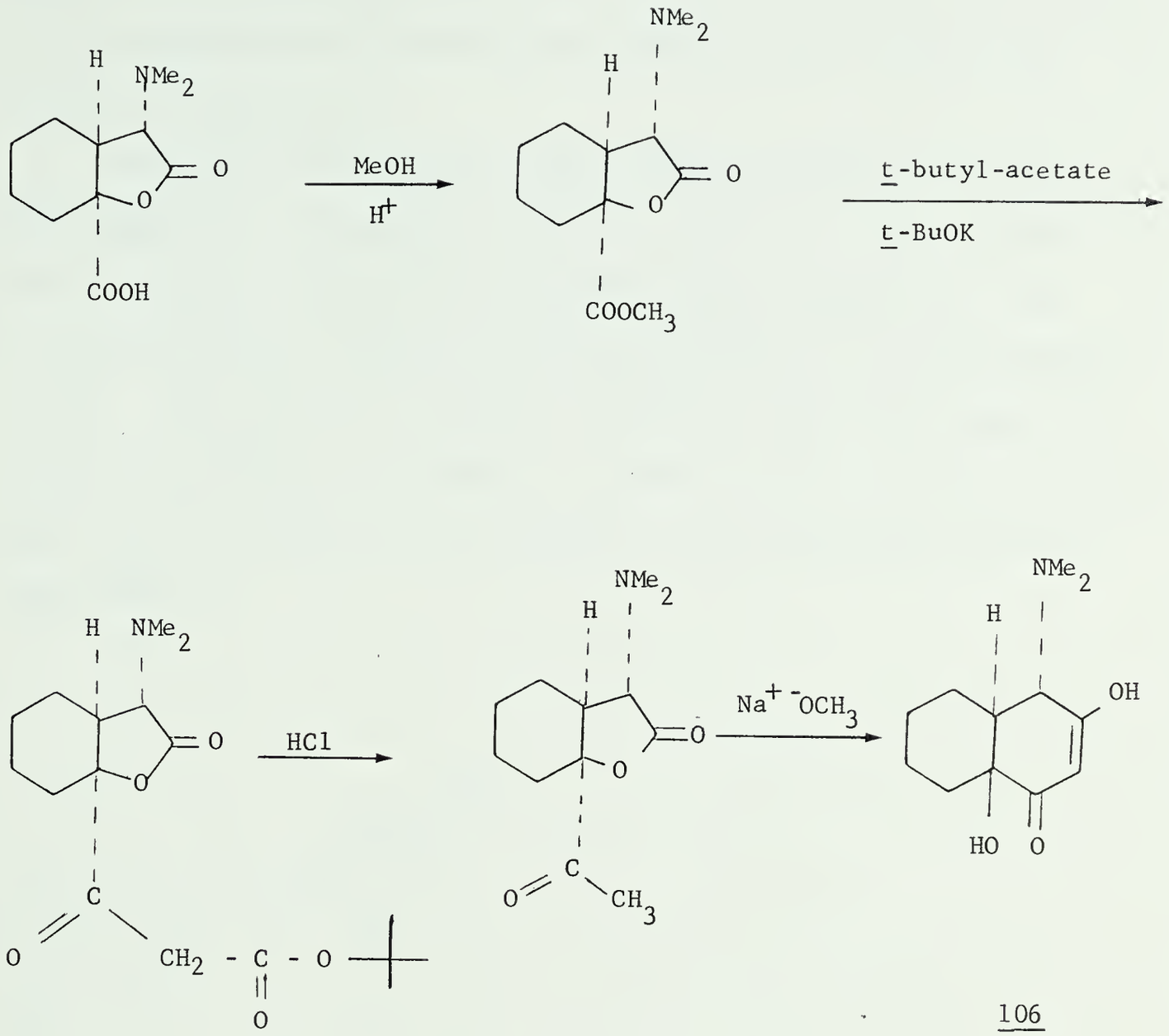
Scheme 8: Synthesis of cis-4-dimethylaminodecalol-9-dione 1,3 (106)



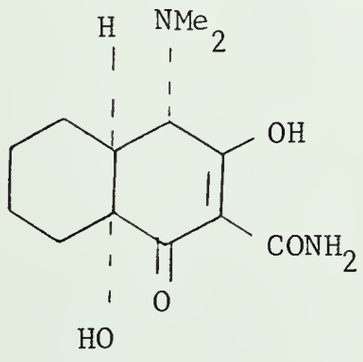
98 99 100

101 102 103

104 105


H
NMe2
O
O
COOH
MeOH
H+
NMe2
H
O
O
COOCH3
t-butyl-acetate
t-BuOK
H
NMe2
O
O
C
O
CH2 - C - O
O
HCl
NMe2
H
O
O
C
O
CH3
Na+ -OCH3
NMe2
H
OH
HO
O

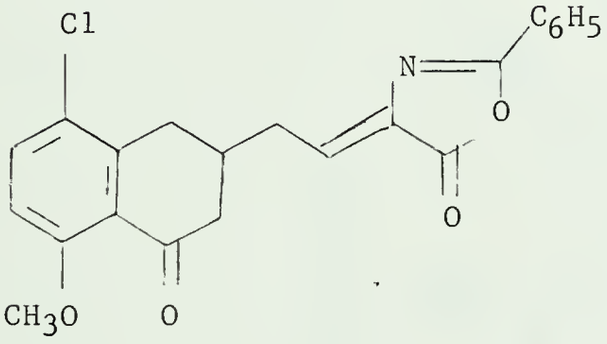
106


H
NMe2
OH
CONH2
O
HO

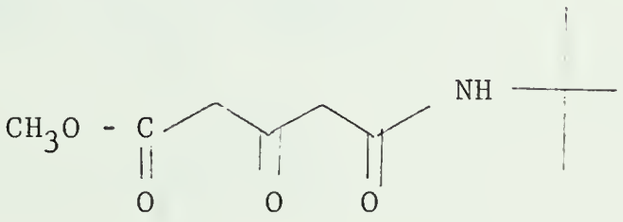
107

In the total synthesis of 6-demethyl-6-deoxytetracycline (40) by Muxfeldt, the construction of the AB rings was accomplished in one step by the condensation of the unsaturated azlactone 64 with the active methylene compound, 65.

When simpler azlactones are used, A-ring analogs are produced. For example[78], azlactone 108 was allowed to react with the potassium salt of dimethyl-3-oxoglutarate 109 to form 110 in high yield. The 1,3-cyclohexanedione compound 110 could be saponified and decarboxylated to 111. Alternatively, if 110 was treated with Meerwein's reagent and the reaction mixture was subjected to aqueous work-up, the amino compound 113 could be obtained without isolation of the intermediate imino ether 112 (Scheme 9).

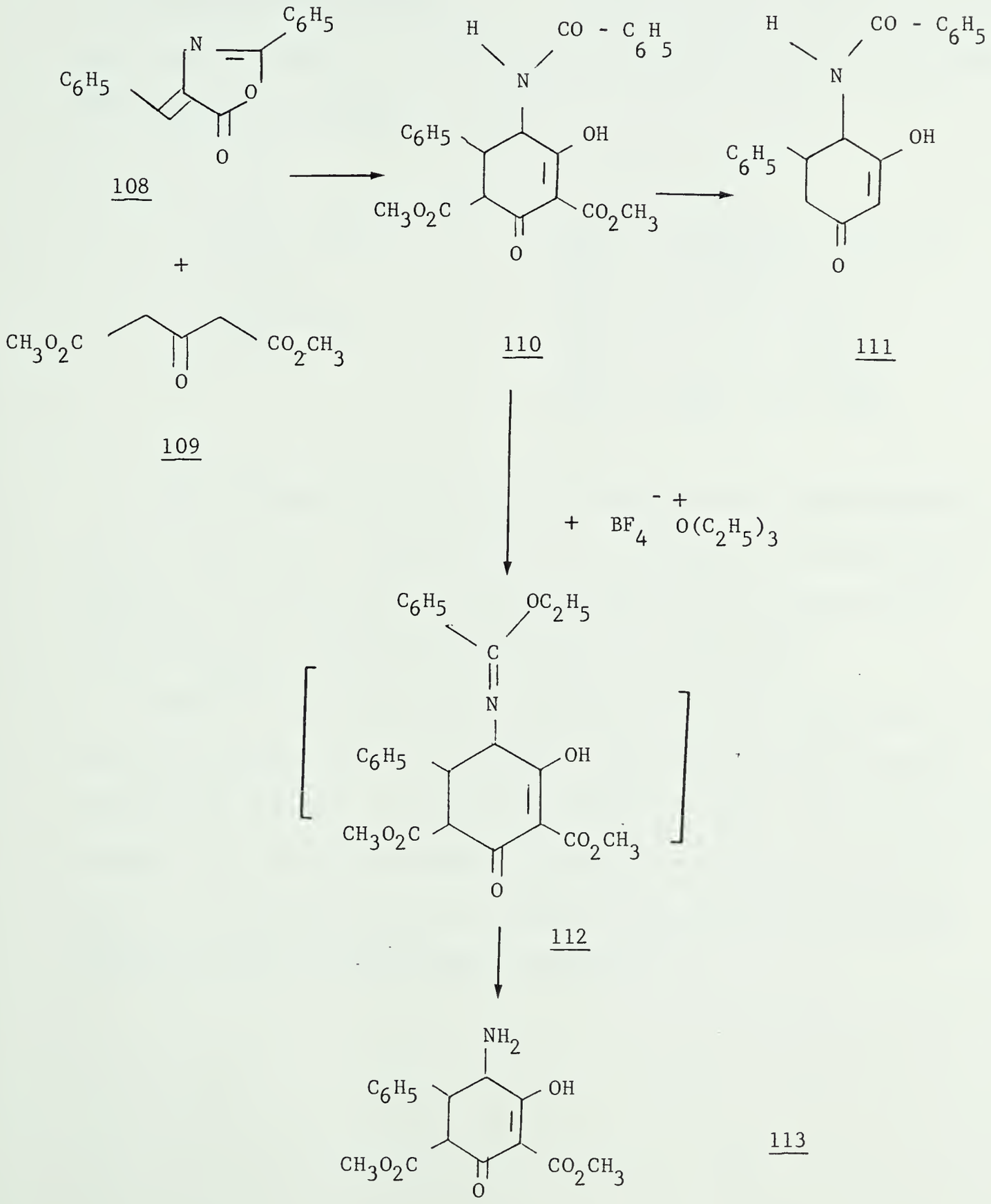


64

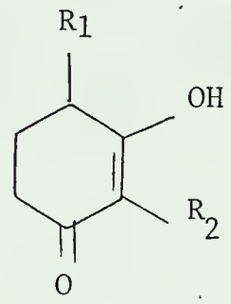


65

Scheme 9: Synthesis of Ring-A Analogs by Muxfeldt



The synthesis of A-ring analogs took a different turn in the hands of K. Tomino[79,80]. The key to this synthetic scheme lies in the catalytic hydrogenation of substituted resorcinols to yield the corresponding 1,3-cyclohexanedione derivatives. Catalytic hydrogenation of resorcinol was reported as early as 1934[81], and optimum conditions for the reduction were reported by Thompson in 1947[82].



114 $R_1 = NHCOC_6H_5$, $R_2 = H$.

115 $R_1 = N(CH_3)_2$, $R_2 = H$.

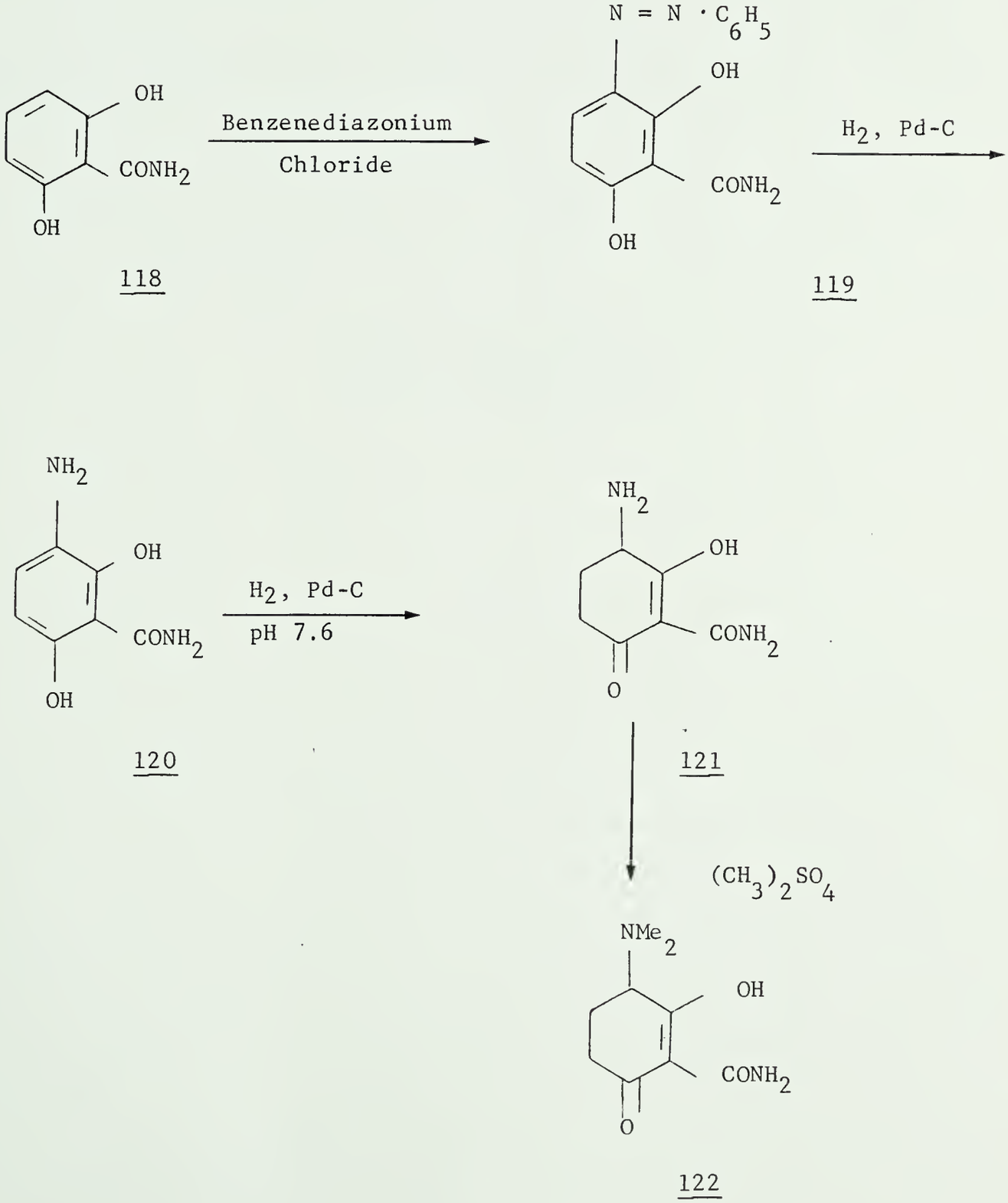
116 $R_1 = H$, $R_2 = CONH_2$.

117 $R_1 = NH_2$, $R_2 = CONH_2$.

The 4-benzamido compound 114 was prepared in this investigation by an alternate route. Compound 116 was also prepared as a part of this study by the reaction of 1,3-cyclohexanedione with potassium cyanate using Muxfeldt's method of carboxamidation of dimedone[75].

Tomino[80] also reported the synthesis of compound 122, which of course was the synthetic goal of this project. The amino resorcinol 120 was catalytically reduced at atmospheric hydrogen pressure with 10% palladium-charcoal at 40-50° after careful adjustment of reaction alkalinity to pH 7.6. As mentioned earlier, attempts at duplicating the reduction process[7] in this laboratory were unsuccessful.

Scheme 10: Synthesis of 2-Carboxamido-4-dimethylamino-1,3-cyclohexanedione (122) by Tomino

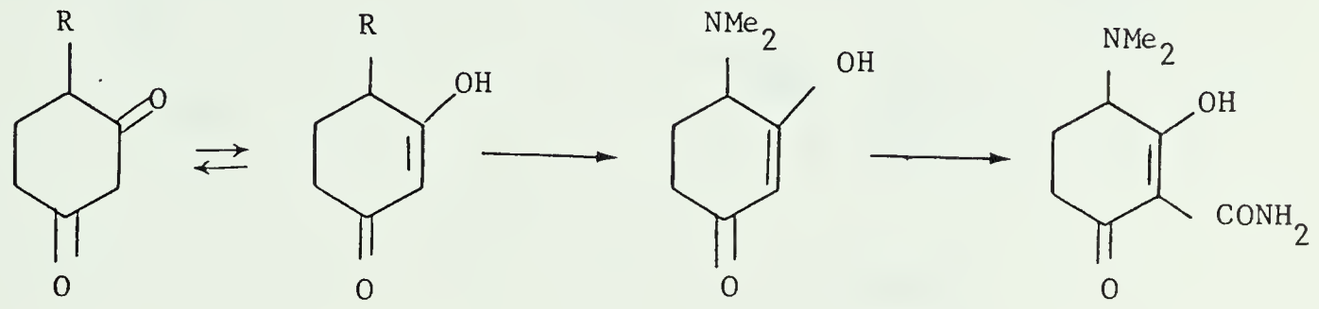


118 119 120 121 122

RESULTS AND DISCUSSION

A straightforward general approach at obtaining the desired ring A analog appeared to be the one shown in scheme 11. Starting with an appropriately substituted 1,3-cyclohexanedione (123), conversion to the dimethylamino compound 124, followed by introduction of the 2-carboxamide group would give the desired compound 122.

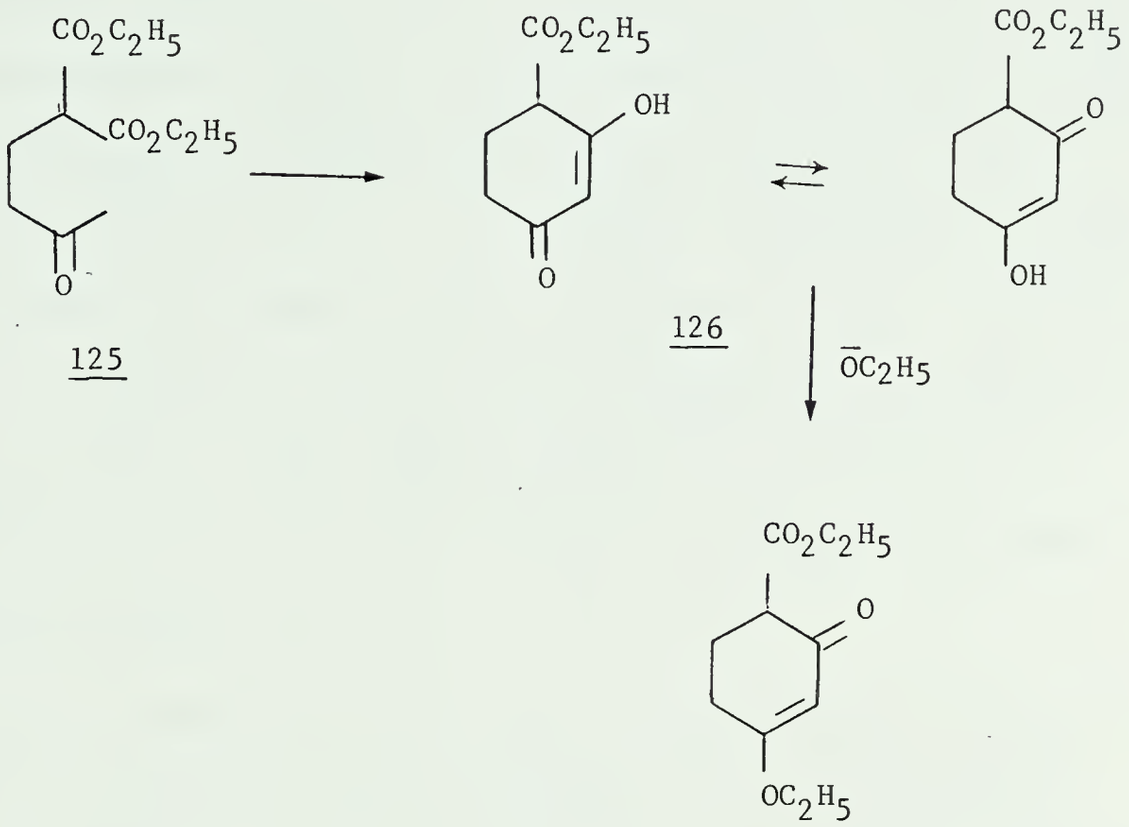
Scheme 11: General Route to Desired Compound 122



123 124 122

Introduction of the dimethylamino group was envisaged via the ester, employing the Curtius rearrangement. Accordingly, the synthesis of the previously reported[83] 4-carboethoxycyclohexane-1,3-dione (126) was undertaken. This compound was prepared by the cyclization of the keto-ester 125. The original authors employed sodium ethoxide in ethanol to effect the cyclization and obtained variable yields, often isolating the enol ether 127 as the major product. Formation of 127 was presumed to arise from the further reaction of ethoxide ion on the cyclized product as depicted in scheme 12. In order to avoid this side-product, the cyclization in this laboratory was accomplished with sodium hydride in anhydrous benzene in high yield, and 126 was the only

Scheme 12: Formation of 6-Carboethoxy-3-ethoxy-2-cyclohexen-1-one (127)

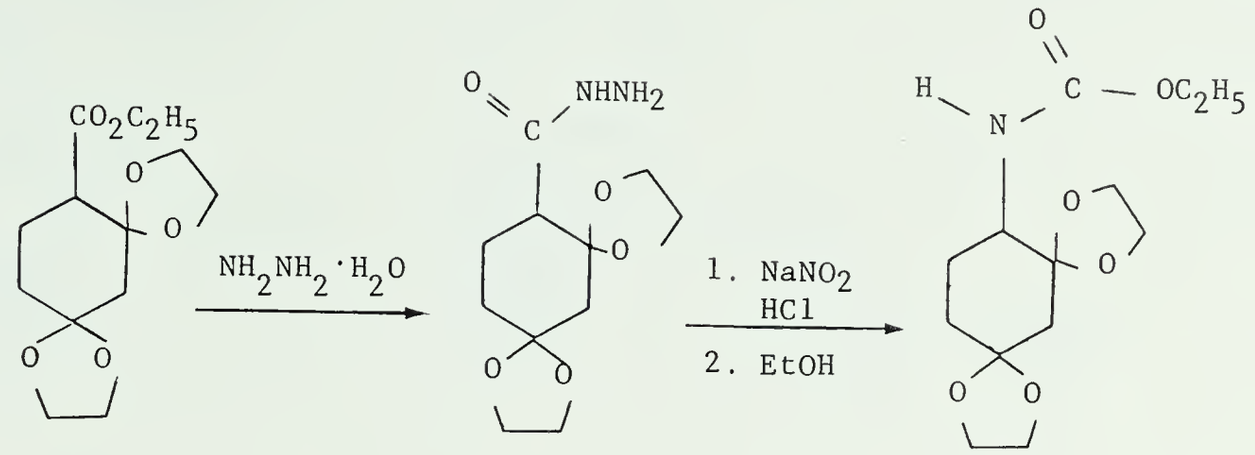


product isolated. An interesting feature of the nmr spectrum of 126 was the two overlapping triplet-quartet ethyl systems separated by less than 0.1 δ . This finding is consistent with proton tautomerism as depicted in scheme 12 for this compound.

Ketalization of 126 was carried out in the usual manner in ethylene glycol and benzene with a catalytic amount of p-toluenesulfonic acid to give compound 128. The nmr spectrum displayed a strong singlet at δ 4.0 which accounted for the eight protons of the two ethylene ketals at C-1 and C-3. The characteristic methyl triplet and methylene quartet

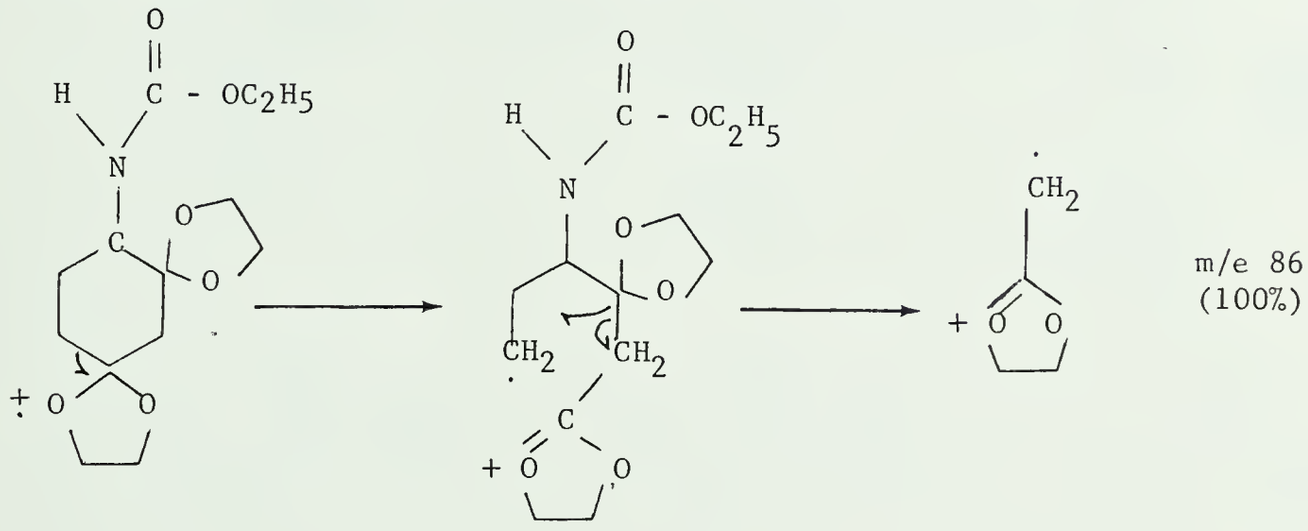
of the ethyl ester were also present but there was no evidence of the overlapping ethyl systems observed in 126. Crude 128 was used without further purification in the next reaction in which it was treated with hydrazine hydrate to form the hydrazide derivative 129. The hydrazide was very water soluble and could not be extracted from its aqueous solution by the usual organic solvents (e.g. ether, benzene, chloroform, etc.). Its successful crystallization required a thoroughly dried sample as well as anhydrous solvent. The infrared spectrum showed three distinct N-H stretching bands of medium-to-strong intenstiy at 3400, 3300 and 3200 cm^{-1}. The region from 1800-1680 cm^{-1} was clear which indicated the absence of the ester carbonyl grouping.

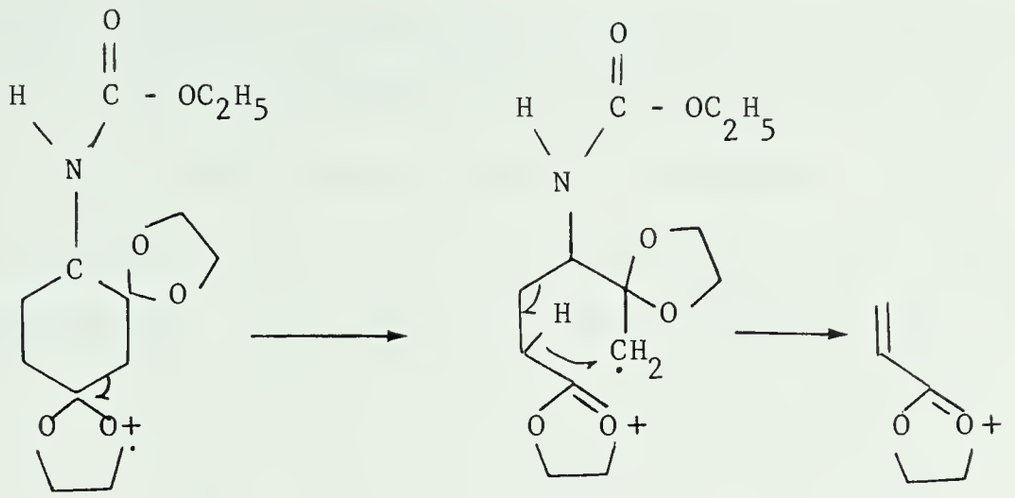
The rearrangement of acyl azides in inert solvents such as benzene or chloroform to give isocyanates was first reported by Curtius in 1890[84]. Acyl azides were prepared by treating a hydrazide in cold aqueous solution with nitrous acid. The isocyanates could either be isolated or the azides were allowed to rearrange in alcohol or water so that the intermediate isocyanates were converted to urethans or ureas. Since urethans are normally stable, crystalline compounds, the ethyl urethan 130 was chosen as the intermediate point in the conversion of the hydrazide to the amine. The mass fragmentation pattern (scheme 13) is consistent with this structure. Similar fragmentation was evident for the related diketal hydrazide 129.



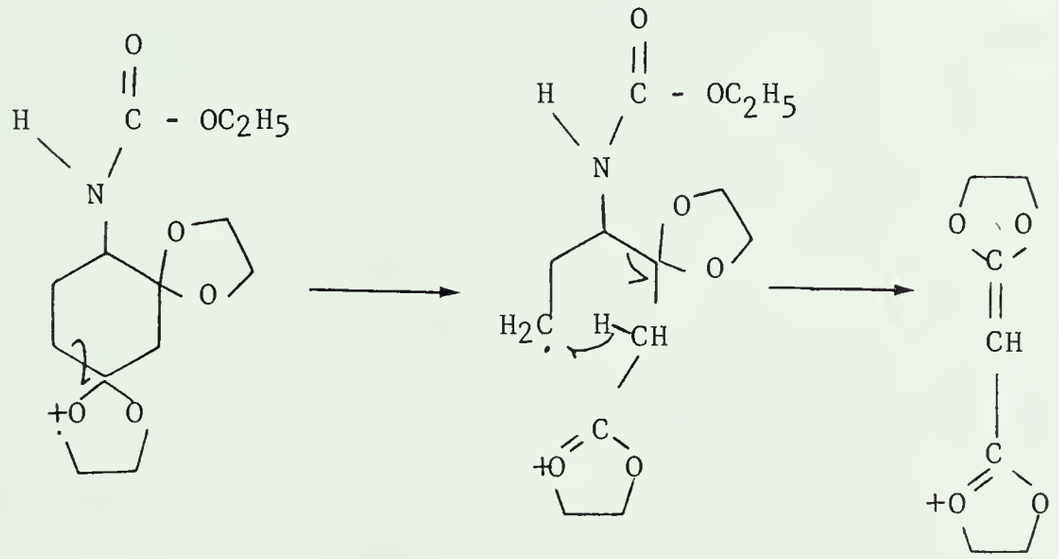
128 129 130

Scheme 13: Mass Fragmentation Pattern of 130.

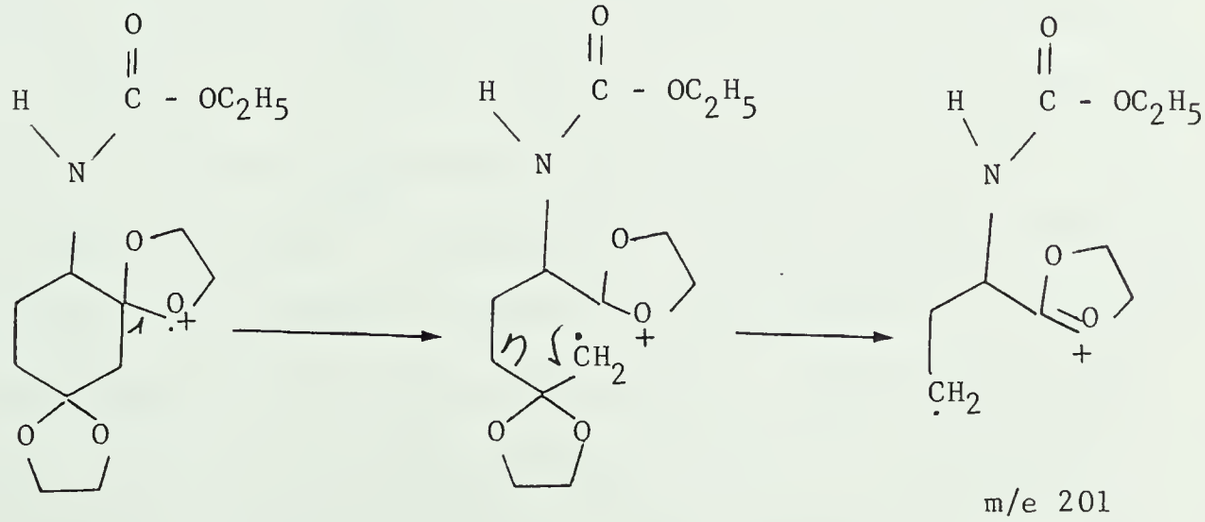




m/e 99
(54%)



m/e 157
(73%)



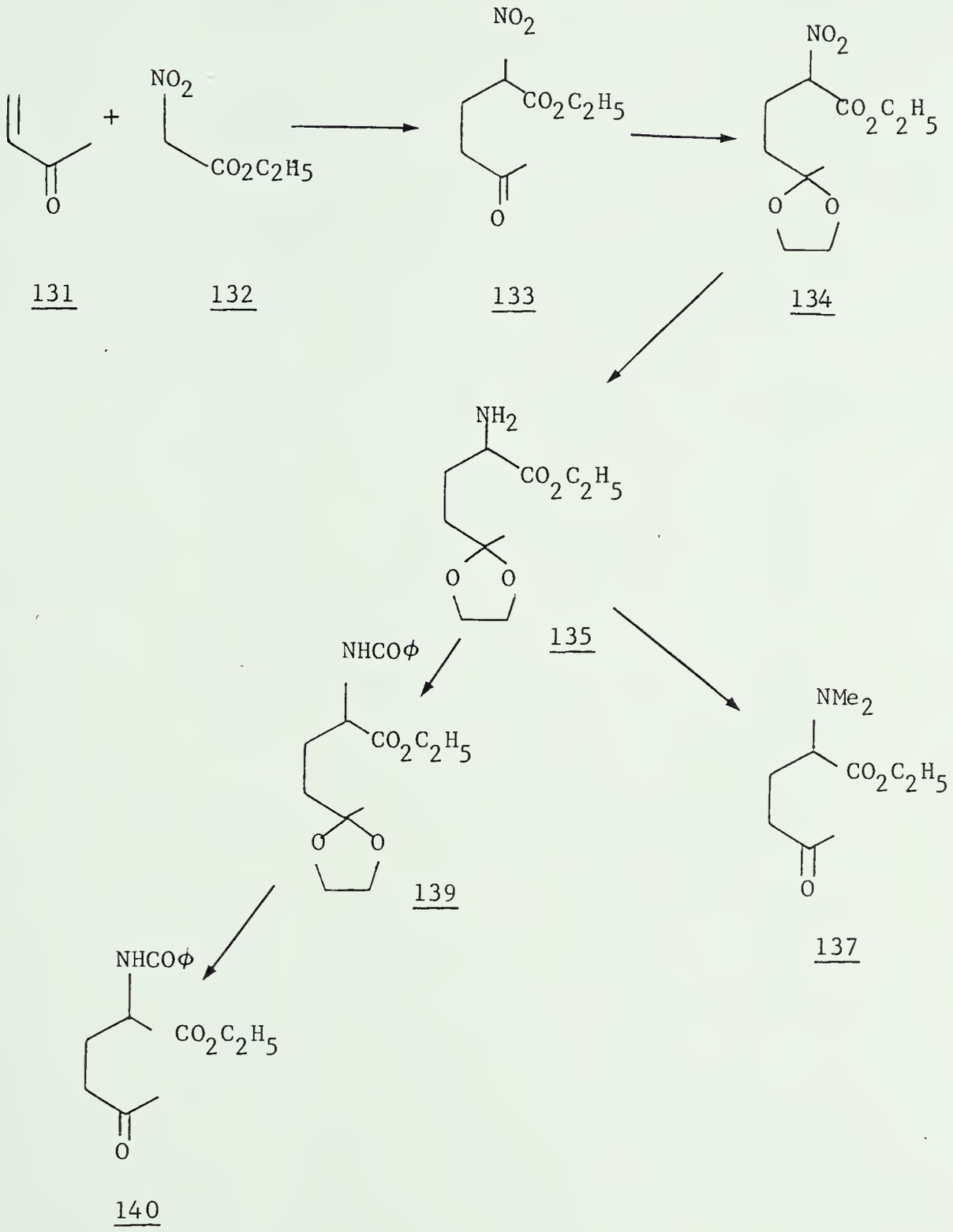
m/e 201
(64%)

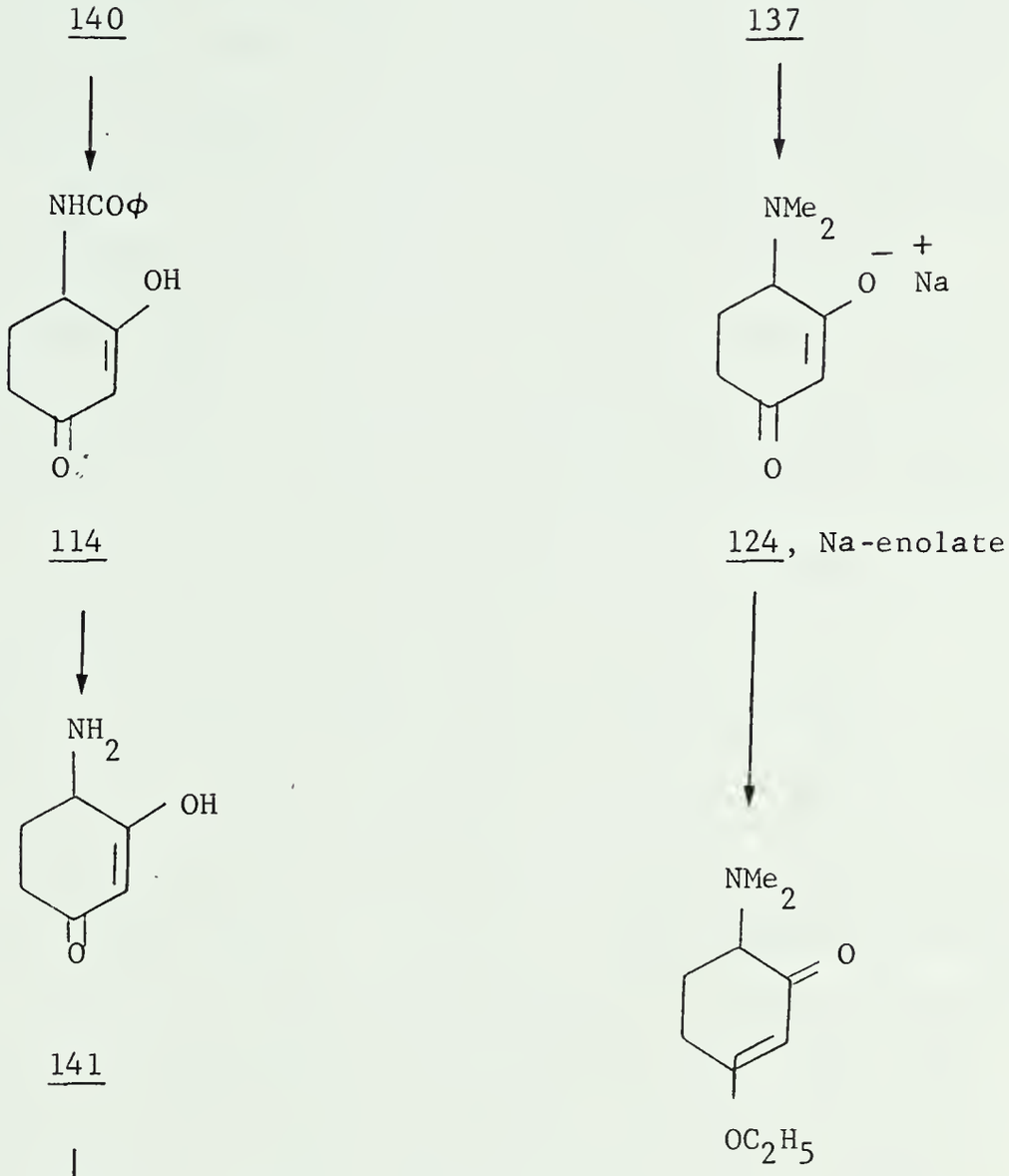
The common procedure for hydrolyzing urethans to the corresponding amines is to heat the urethan with concentrated hydrochloric acid under reflux or in a sealed tube at elevated temperatures. Alkaline hydrolysis could also be used with either alkali metal hydroxides or barium hydroxide. Preliminary experiments indicated that acid hydrolysis of 130 proceeded much faster than base hydrolysis. Several trials were attempted employing concentrated hydrochloric acid and various reaction times. In all cases the hydrochloride salt could not be isolated, and the reaction products were very difficult to purify. It appeared that the β-dicarbonyl system in the desired product was too labile[78,85] to endure the strong acidic conditions necessary to bring about the hydrolysis of the ethyl urethan. Since investigations into another route which would yield the same compound were well underway, and had shown promise, no further work was done on the urethan hydrolysis.

In the alternate route, the amino function at C-4 was introduced by catalytic hydrogenation of the nitro group, affording the key intermediate 135, by the sequence of reactions shown in scheme 14.

Ethyl nitroacetate (132) was prepared by the method of Rodionov[86]. Addition of this active methylene compound to the activated double bond in methyl vinyl ketone (131) could be effected with Triton B[61]. More recently White and Baizer[87] introduced the use of tri-n-butyl-phosphine as a catalyst in the Michael addition of 2-nitropropane to a number of activated olefins such as methyl vinyl ketone. Application of their technique to the addition of 132 to 131 resulted in a considerably improved yield of compound 133 (78%). Before reduction of the nitro group could be carried out the keto function in 133 was protected from

Scheme 14: Synthesis and Elaboration of Ethyl-2-amino-5-(1,2-dioxanyl)-hexanoate (135).




140
NHCOφ
OH
O
114
NH2
OH
O
141
124
137
NMe2
O
Na
O
124, Na-enolate
NMe2
O
OC2H5
138

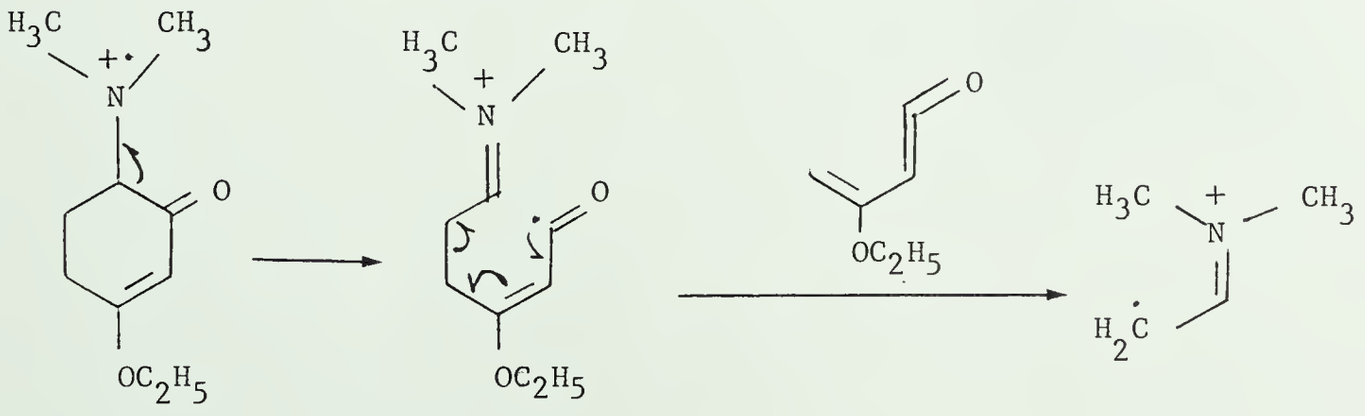
concomitant reduction by ketal formation using ethylene glycol in benzene, with p-toluenesulfonic acid as the catalyst under a Dean-Stark trap. Attempted reduction of the nitro group to the primary amine function with hydrogen and 10% palladium-on-charcoal failed at low pressures. However, at a pressure of 1,000-1,500 psi, with the same catalyst, hydrogenation proceeded smoothly to afford the corresponding amine 135 in 93% yield. In the nmr spectrum of 135, from δ 1.0-1.5, there were four peaks integrating for six protons. The first and the fourth peaks were both separated from the second peak by 7 Hz. Accordingly, these three peaks were assigned to the triplet of the methyl of the ethyl ester. The third peak at δ 1.28 was assigned to the terminal methyl α to the carbon bearing the 1,2-dioxanyl group. At δ 2.25, a relatively broad peak, capable of deteurium exchange and integrating for two protons was present. This peak was assigned to the primary amine function. The ketal methylenes appeared as a singlet at δ 3.90, followed immediately by a quartet centered at δ 4.17 arising from the methylene group of the ethyl ester. The infrared spectrum (solid state) showed medium absorption bands at 3200 and 3060 cm^{-1} attributable to the N-H stretching frequencies of the amino function. The amine 135 could be reductively alkylated in excellent yield by treatment with excess formaldehyde and hydrogen at a pressure of 40 psi using 10% palladium-on-charcoal as the catalyst[51,88]. The ketal protective group was removed in the usual manner by acid hydrolysis and the compound was characterized as the ketone 137. The nmr spectrum of the keto dimethylamino ester 137 showed singlets at

δ 2.13 and 2.30, integrating for three and six protons, respectively. The first singlet was assigned to the keto methyl protons and the latter singlet was assigned to the N-dimethyl amino protons. When cyclization was attempted with the keto dimethylamino ester 137 using sodium hydride in anhydrous benzene, an amorphous precipitate was obtained after refluxing for ten hours. A qualitative uv spectrum was recorded by dissolving the solid precipitate in ethanol. Its λ_{max} was 282 nm which was shifted to 260 nm (with reduced absorption intensity) when a drop of hydrochloric acid was added. A sample of dimedone (96) behaved similarly, in that in basic solution its λ_{max} was 284 nm which was shifted to 260 nm when acid was added. The above uv experiment indicated that the solid precipitate was the sodium salt of 3-hydroxy 4-dimethylamino-2-cyclohexen-1-one (124). Therefore, dilute hydrochloric acid was added to destroy unreacted sodium hydride and to convert the sodium salt to its hydrochloride. The acid solution was then brought to pH 8-9 with 5% $NaHCO_3$. However, extraction with diethyl ether failed to afford the desired compound 124. At this point the aqueous solution was made acidic (pH$\simeq$2) and evaporated to dryness in vacuo. The resultant mass was extracted with absolute ethanol, and the solid remaining after evaporation of the ethanol was dissolved in water and the solution basified with $NaHCO_3$ solution. Extraction with chloroform yielded a liquid which was then purified by tlc. Spectral analysis pointed to the structure illustrated as 138, comparing favorably with the spectra of the structurally related 6-carboethoxy-3-ethoxy-2-cyclohexen-1-one (127) referred to earlier. The infrared spectrum of compound 138 showed intense absorption bands at 1650 and 1600 cm^{-1}, as did that of compound 127

(1650 and 1602 cm^{-1}). The nmr spectrum of 138 showed a prominent singlet at δ 2.4, which integrated for six protons and was assigned to the two methyls of the dimethylamino group. A second singlet appeared at δ 5.3, integrating for one proton and was assigned to the olefinic proton. The presence of the ethoxy group was evident as there was a 3-proton triplet at δ 1.33 and a 2-proton quartet at 3.85. Again these data compared closely to those reported for 127 (singlet at δ 5.30, quartets centered at δ 4.20 and 3.90, and triplets at δ 1.4 and 1.3)[83]. Of course the nmr spectrum of 127 had an extra system due to the carboethoxy group.

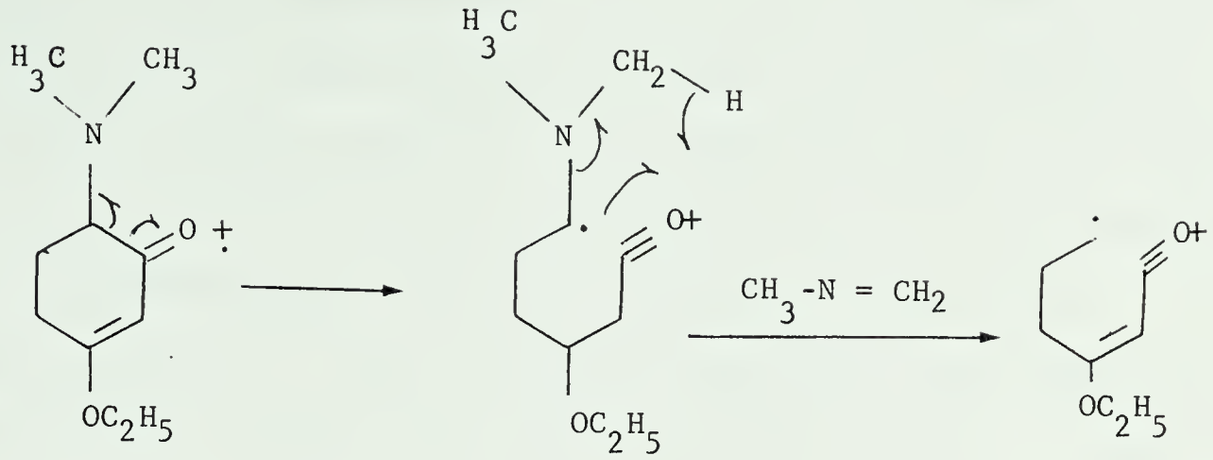
Its structure was established unequivocally by exact mass measurement. The mass of the molecular ion in its mass spectrum was 183.1255, which was precisely what the exact mass should be. Exact mass measurement of fragment ions suggested a possible fragmentation pattern as shown in scheme 15.

Scheme 15: Mass Fragmentation Pattern of 6-Dimethylamino-3-ethoxy-2-cyclohexen-1-one (138).



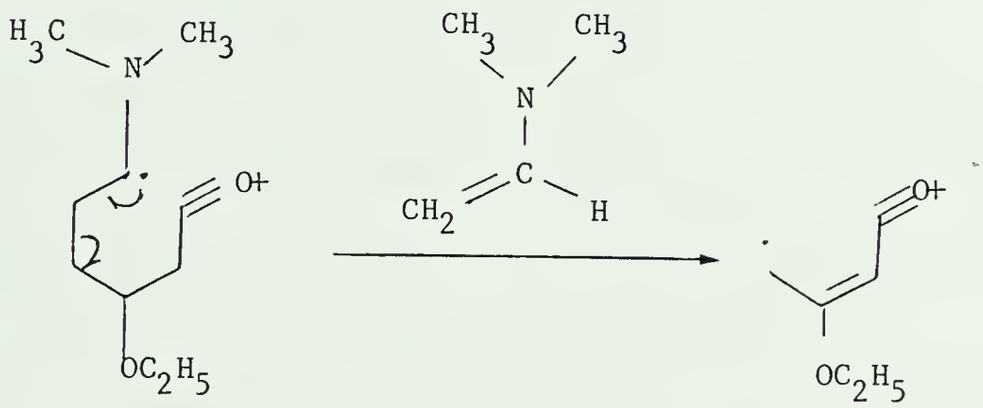
138

C_4H_9N, m/e 71.0724
(100% rel. abund.)



$C_8H_{12}O_2$,

m/e 140.0833
(32.6% rel.abund.)



$C_6H_8O_2$,

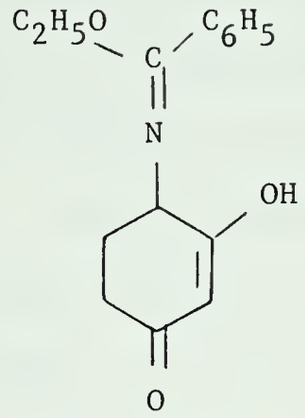
m/e 112.0518
(5.9% rel.abund.)

The problems encountered in isolating compound 124 appeared to be associated with the amphoteric nature of this compound. Therefore, a new approach involving conversion of the basic amino moiety to a neutral species, followed by cyclization and subsequent removal of the protective group was embarked upon. The benzamido group was chosen, since Muxfeldt reported[62,67] that it could be removed under mild conditions with the use of triethyloxonium tetrafluoroborate (Meerwein's reagent)[68]. Hence, synthesis of compound 114 was projected (scheme 14) following which carboxamidation could be attempted with one of several available reagents. Accordingly, the amino ketal ester 135 was converted to the benzamido derivative 139 by treatment with benzoyl chloride. Compound 139 could be deketalized readily by acid hydrolysis at room temperature to give the corresponding oxo compound 140.

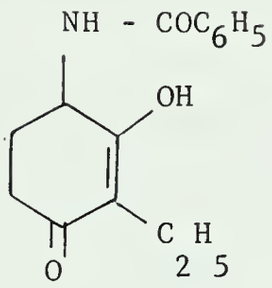
Crude 140 was used in the next step without further purification. Its cyclization with sodium hydride and anhydrous benzene gave a white solid which melted at 175-177°C. This compound 114 was previously synthesized by Tomino employing a different approach[79].

With 4-benzamido-cyclohexane-1,3-dione (114) at hand, removal of the benzoyl protective group was attempted employing triethyloxonium tetrafluoroborate, as described by Muxfeldt[89]. The compound obtained had a molecular formula of $C_{15}H_{17}NO_3$ (exact mass spectrum) as opposed to the expected $C_{13}H_{13}NO_3$, a difference of C_2H_4. The fact that triethyloxonium tetrafluoroborate was an alkylating agent limited the possible structures to 142, 143, or 144.

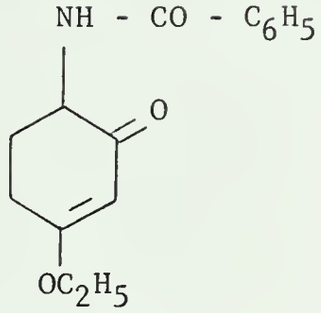
Structure 142 would be the imino ether intermediate before hydrolysis with potassium bicarbonate (or sodium acetate). However, the isolated



142



143



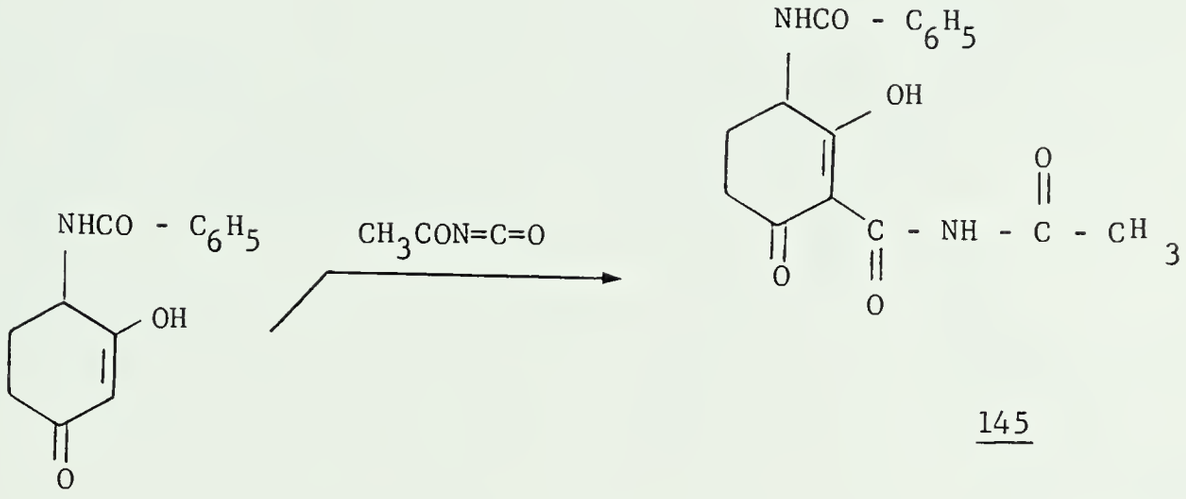
144

compound survived hydrolysis under various mild basic or acidic conditions with reaction times as long as twenty-four hours. Imino ethers would be expected to be readily hydrolyzed under these conditions in a very short time. Furthermore, the nmr spectrum did not show any sign of an enolic hydroxyl proton. The compound showed an olefinic proton at the same chemical shift (δ 5.3 - δ 5.4) as the olefinic proton in similar systems, such as compound 114 and 138. Accordingly, it appeared that the correct structure was 144, which arose from O-alkylation of either one of the oxygens of the cyclohexane-1,3-dione system. The isolation of the O-alkylated product 144 was in agreement with experiments conducted by Durckheimer who employed the same reagent to effect etherification at the 1 (or 3) position of some tetracyclines[10]. Since the compounds studied by Muxfeldt all contained a substituent at the C-2 position, the attempted removal of the protecting group was curtailed at this time with the idea that it would be attempted after the carboxamido group or a precursor had been introduced at the C-2 position. Perhaps there are some steric and/or electronic factors which would explain

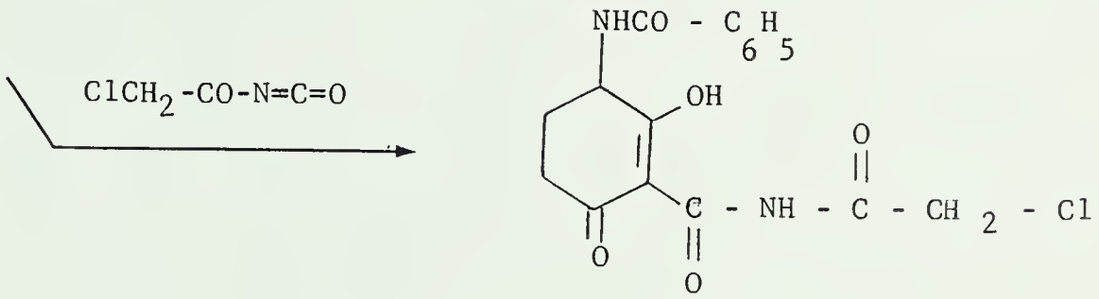
why O-alkylation becomes less significant when there is a substituent at the C-2 position enhancing the reaction involved in the removal of the benzoyl protective group.

Since preliminary carboxamidation experiments on 114 with potassium cyanate failed to yield any useful products, the use of a more reactive reagent, such as the isocyanates, was indicated. The reaction of acetyl isocyanate with the cyclic β-diketone, dimedone (96) to give the corresponding 2-carboxamido derivative had been reported earlier[72-75]. Acetyl isocyanate was first prepared by the reaction of acetyl chloride with silver cyanate[90]. Later it was prepared by reaction of acetyl chloride and isocyanic acid (from thermal decomposition of isocyanuric acid at 600°).[41] These methods were generally quite complex and usually afforded very low yields. A more convenient method of preparing acyl isocyanates was reported by Speziale and Smith[92] from the corresponding amides and oxalyl chloride. Yields with aliphatic amides were very unsatisfactory unless an electron-withdrawing group was present on the α-carbon atom or there were no α- hydrogen atoms. Accordingly, the much more accessible α-chloroacetyl isocyanate[93] appeared to be the reagent of choice which would lead to compound 146 rather than 145.

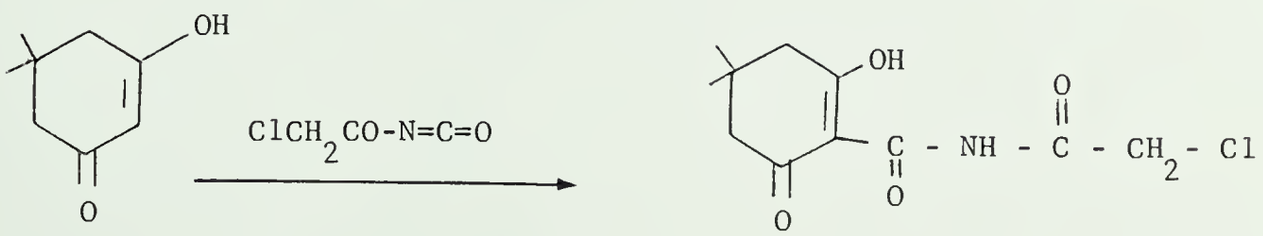
When the sodium enolate of dimedone (96) was allowed to react with α-chloroacetyl isocyanate a yellow solid was obtained. The fact that the product isolated was not the expected N-chloroacetylamide 147 became apparent when the elemental analysis showed that the compound did not contain any chlorine. Also mass spectrometry indicated a composition of $C_{11}H_{13}NO_4$. This, together with the nmr spectrum, reduced the number of possible structures to two, namely compounds 148 or 149 which could arise by the mechanisms proposed in scheme 16.



114 145

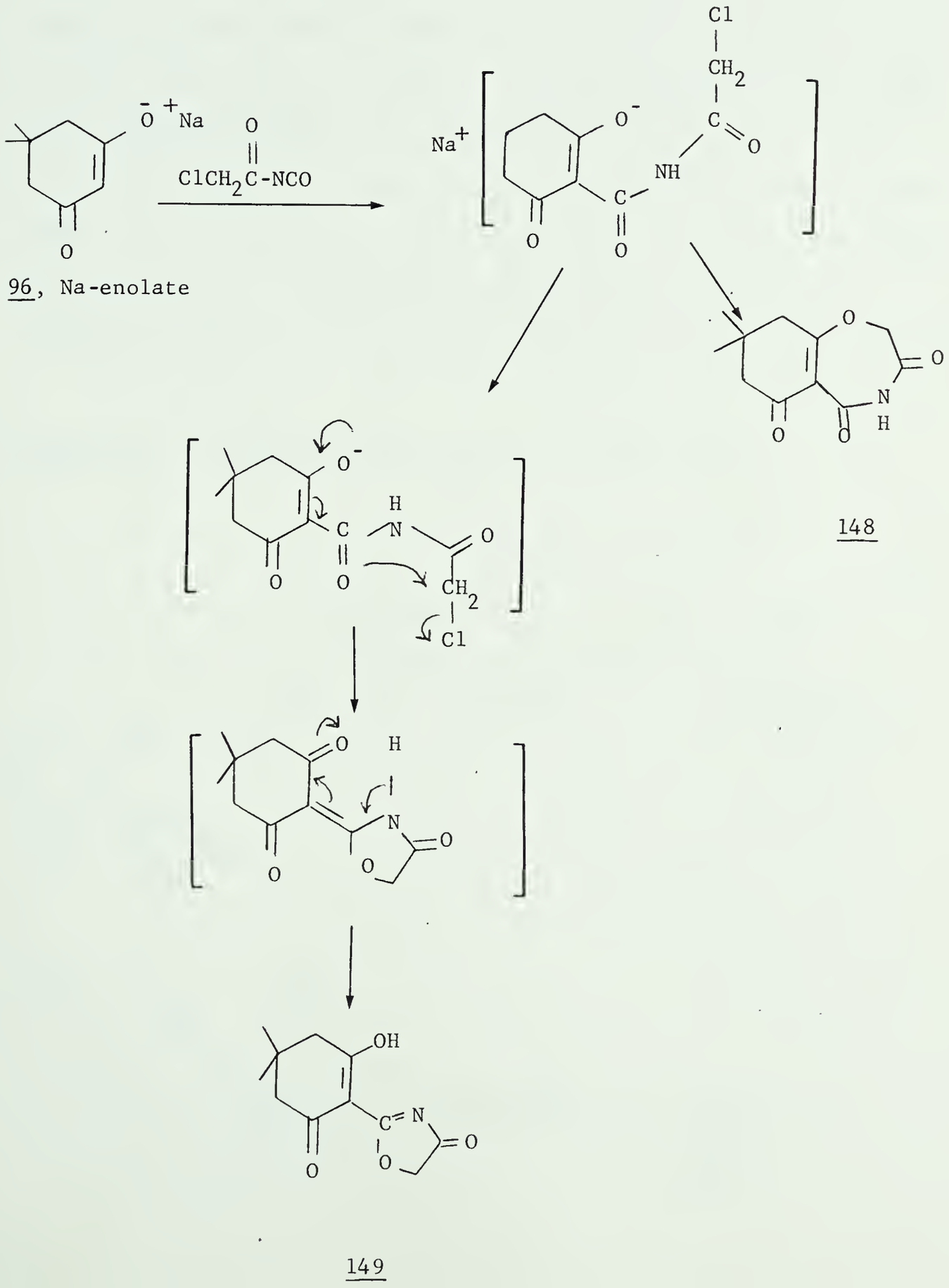


146



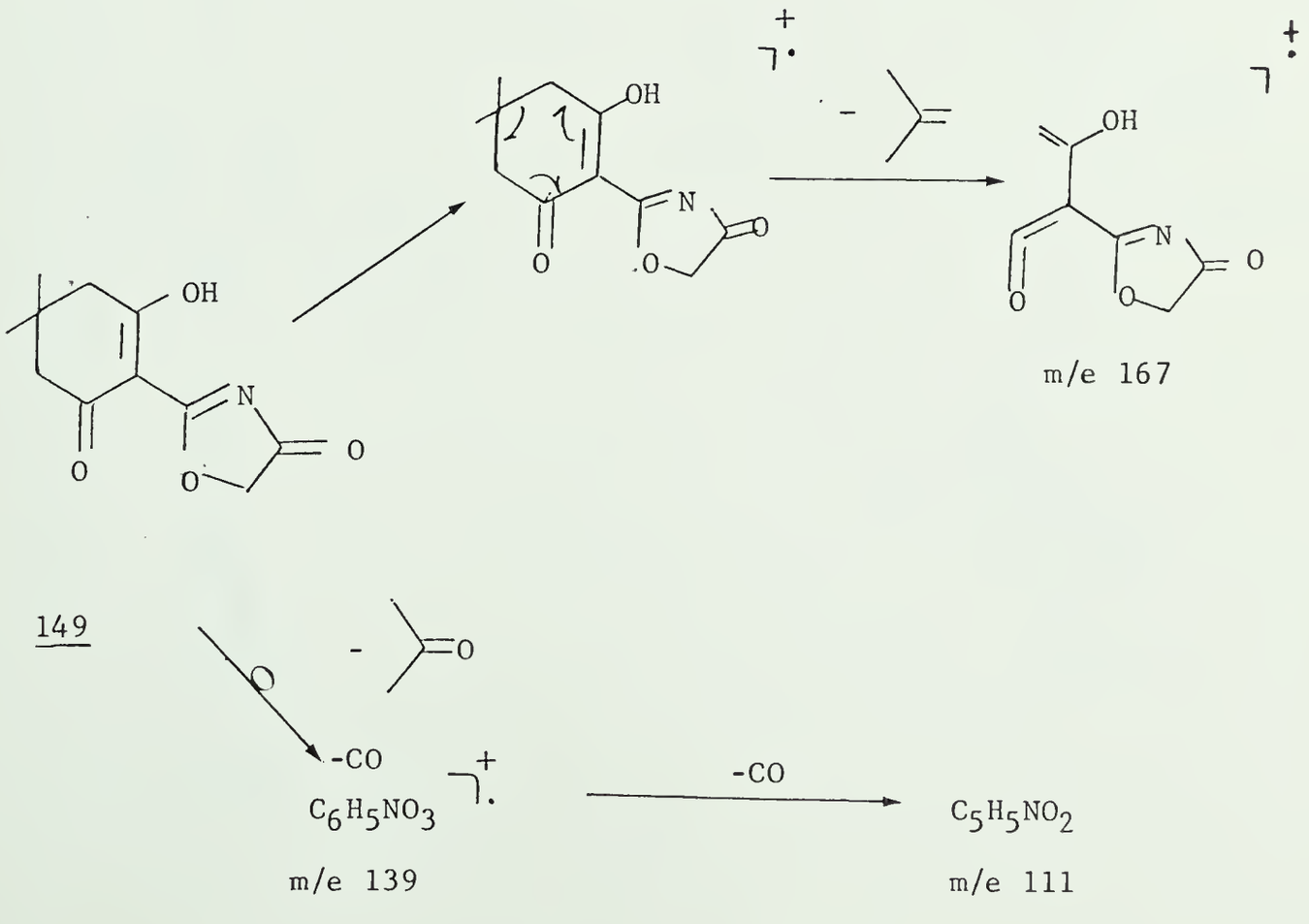
96 147

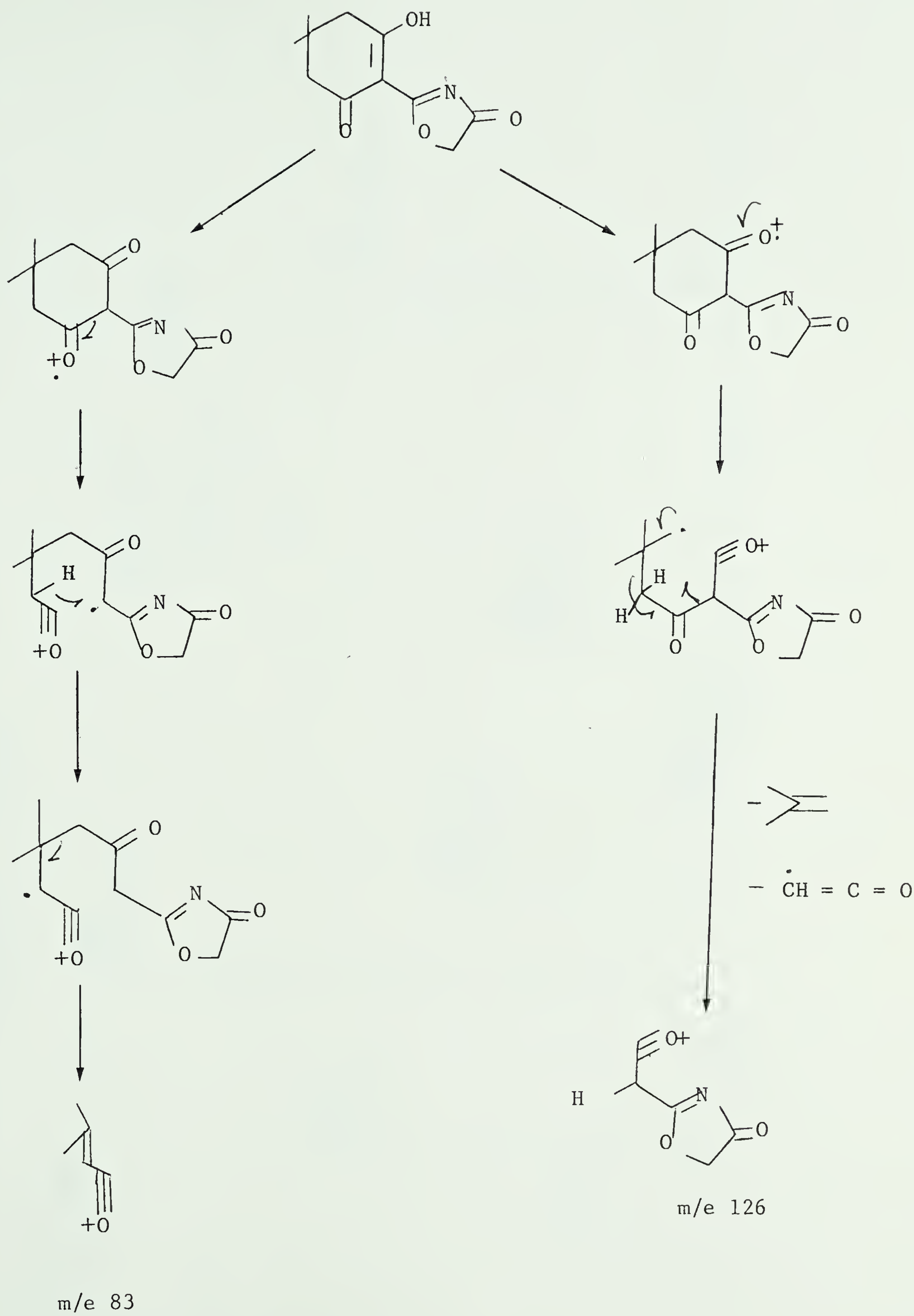
Scheme 16: Proposed mechanism for the formation of 3-hydroxy-5,5-dimethyl-2(2-oxazolin-4-oxo)-2-cyclohexen-1-one (149).



The compound exhibited uv behavior characteristic of an enolic β-diketone system, namely in basic solution a bathochromic shift and a hyperchromic effect (an increase in absorption intensity)[94] were observed which could be reverted by neutralization with acids. This effect would not be expected with compound 148. Fragment ions of its high resolution mass spectrum (Figure 1) further supported structure 149. This compound contained the 2-oxazolin-4-one system, the spectral properties of which have been reported previously[95]. Intense carbon-oxygen and carbon-nitrogen double bond stretching bands at about 1780 and 1575-1499 cm^{-1}, respectively, in the infrared spectra and a methylene singlet at δ 4.6-4.8 ppm in the nmr spectra were characteristic.

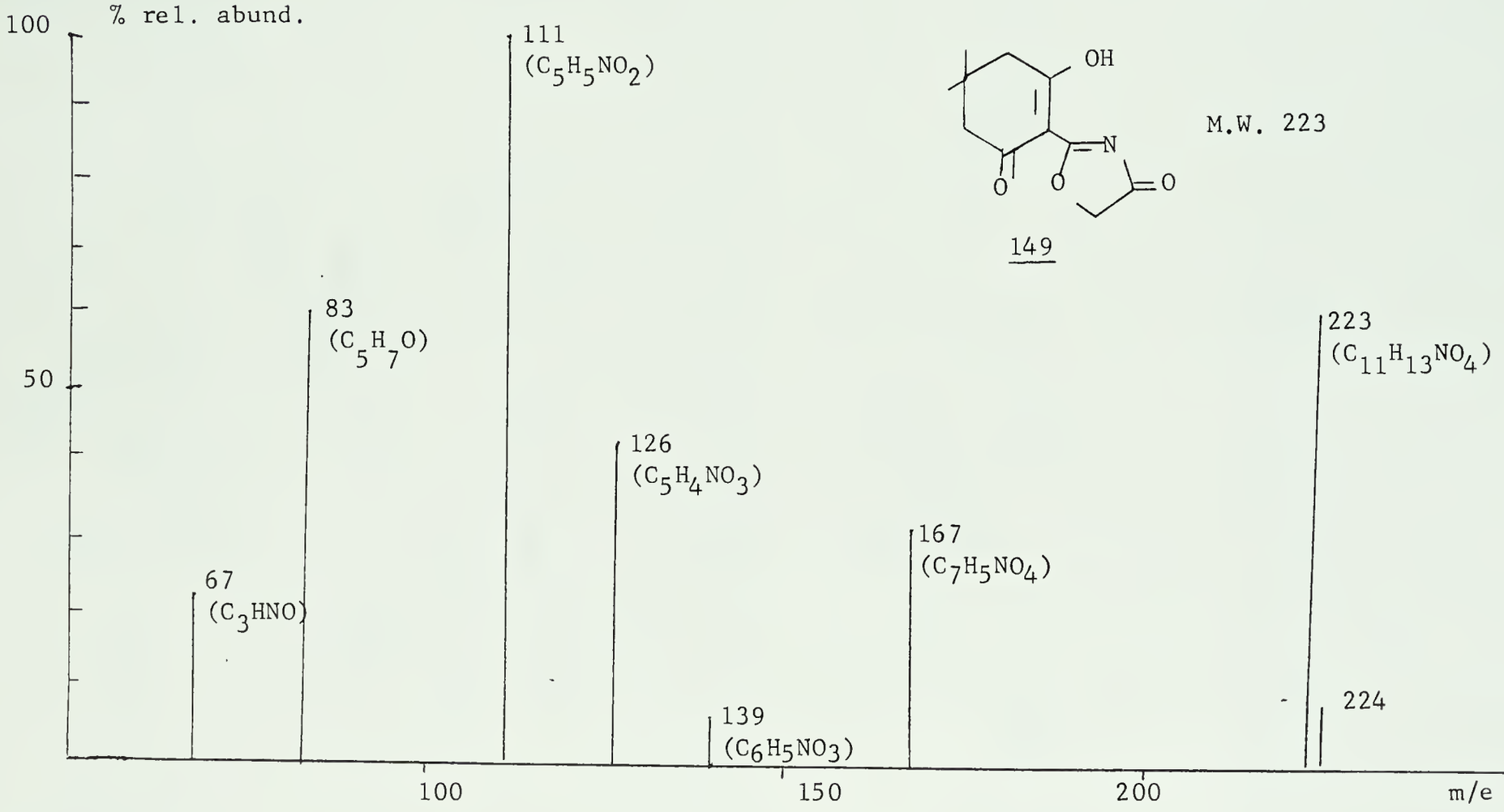
Scheme 17: Mass fragmentation pattern of compound 149.



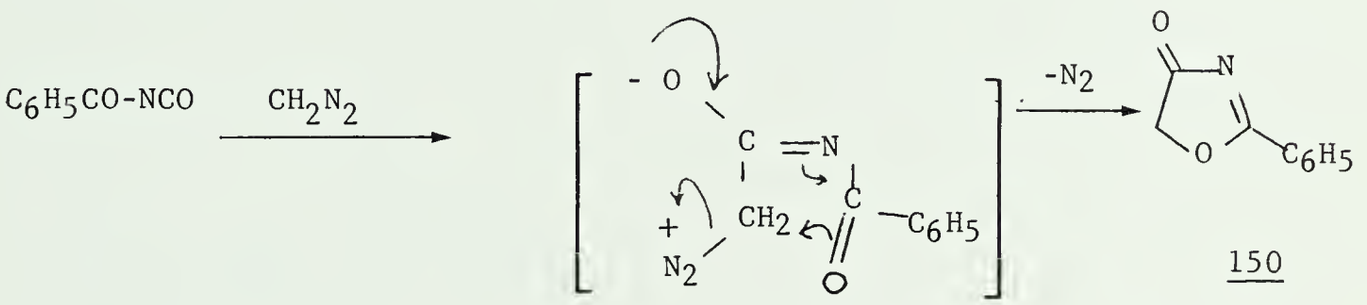


- CH = C = O
m/e 126
m/e 83

Figure 1. Mass Spectrum of 3-hydroxy-5,5-dimethyl-2(2-oxazolin-4-oxo)-2-cyclohexen-1-one (149).

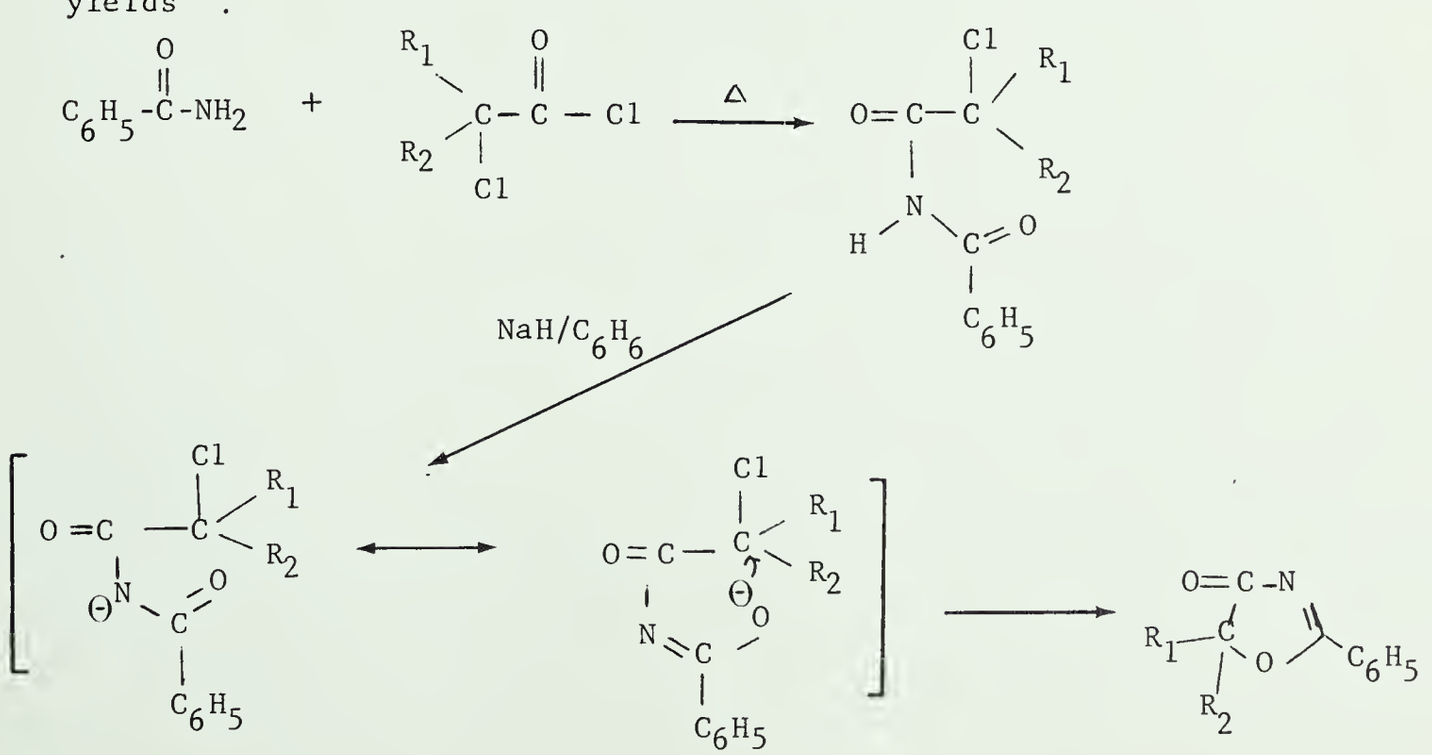


Although compound 149 seemingly represents a considerable departure from the original goal, namely the synthesis of the A-ring of tetracycline, the reaction does provide a novel approach to the synthesis of potentially therapeutically important 2-oxazolin-4-ones. Until 1970, few examples of the 2-oxazolin-4-one system were known[96]. Among these 2-phenyl-2-oxazolin-4-one (150) was relatively well-known and could be prepared from reaction of benzoyl isocyanate with diazomethane[97].



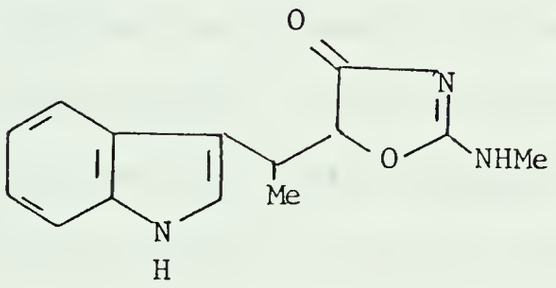
150

A more versatile method making use of benzamide and 2-chloroimides enabled substituted 2-phenyl-2-oxazolin-4-ones 151 to be made in good yields[98].



151

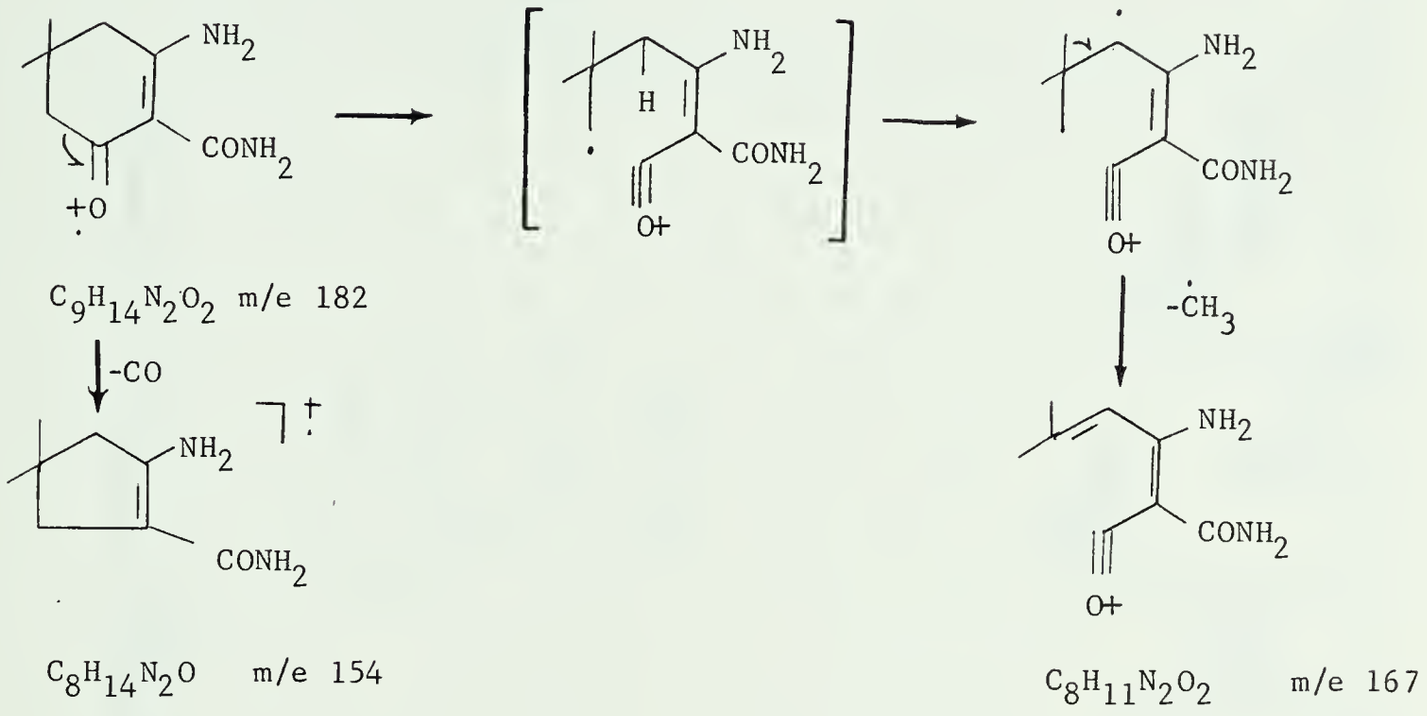
The 2-oxazolin-4-ones as a class have enjoyed growing interest in recent years particularly the 2-amino derivatives which have found use as tranquilizers, antidepressants, memory aids and appetite depressants[99]. The antibiotic indolmycin (152) (produced by Streptomyces griseus) contains the 2-oxazolin-4-one system[100].



152

It was apparent that the 2-oxazolin-4-one system in compound 149 was quite strained (carbonyl at 1770 cm^{-1}). The anticipation that it might collapse under mild conditions prompted further investigation. Hence, compound 149 was treated with a saturated ammonia in methanol solution. The nmr spectrum of the resulting product showed the disappearance of the 2-proton sharp singlet at δ 4.85 that was present in the spectrum of the starting compound. There were three sharp singlets at δ 1.04, 2.4 and 3.07 in the ratio of six to four to two. The singlet at δ 3.07 exchanged with deuterium oxide. The rest of the spectrum consisted of two similar broad peaks at δ 6.77 and 10.57, which were exchangeable with deuterium oxide as well. These features quickly brought to mind the two NH protons of 2-carboxamido-cyclohexane-1,3-dione (and similar systems[101]) which appeared as two broad peaks at δ 6.62 and 9.52. On the basis of nmr alone, several features of the derivative became apparent, namely that it had a primary amide and a primary amino substituent and also a cyclohexane ring system. An accurate mass determination suggested a molecular formula of $C_9H_{14}N_2O_2$. Its UV spectrum displayed a λ_{max} at 260 nm which showed no changes in basic solution. For this reason, the presence of a β-diketone structure was not likely; moreover the molecular formula only allowed for two oxygen atoms, one of which had already been assinged to an amide moiety. Therefore, the only plausible structure was that given by 153. The high resolution mass spectrum (figure 2) and the mass fragmentation pattern (scheme 18) are consistent with this structure. The only perplexing feature preventing a conclusive

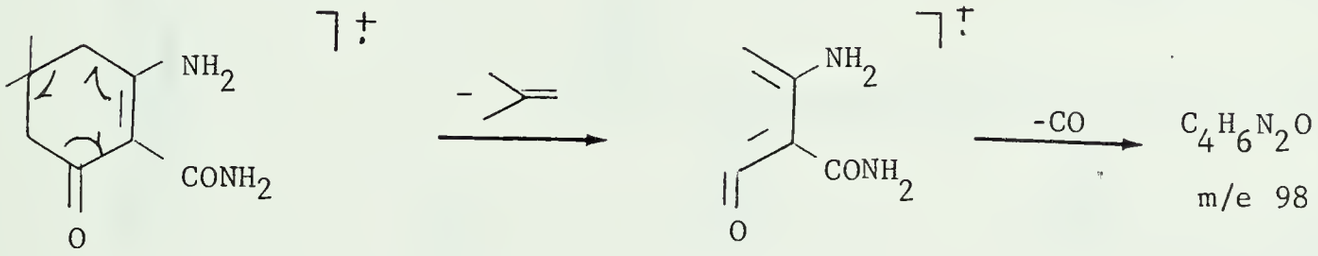
Scheme 18: Mass Fragmentation Pattern of Compound 153.



$C_9H_{14}N_2O_2$ m/e 182

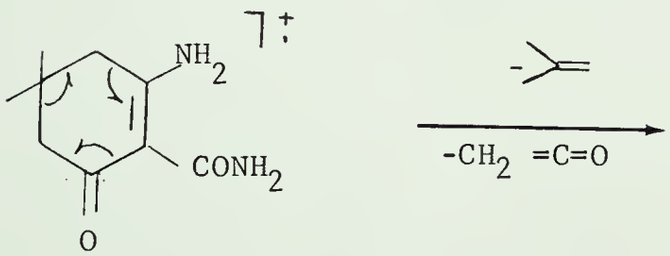
$C_8H_{14}N_2O$ m/e 154

$C_8H_{11}N_2O_2$ m/e 167



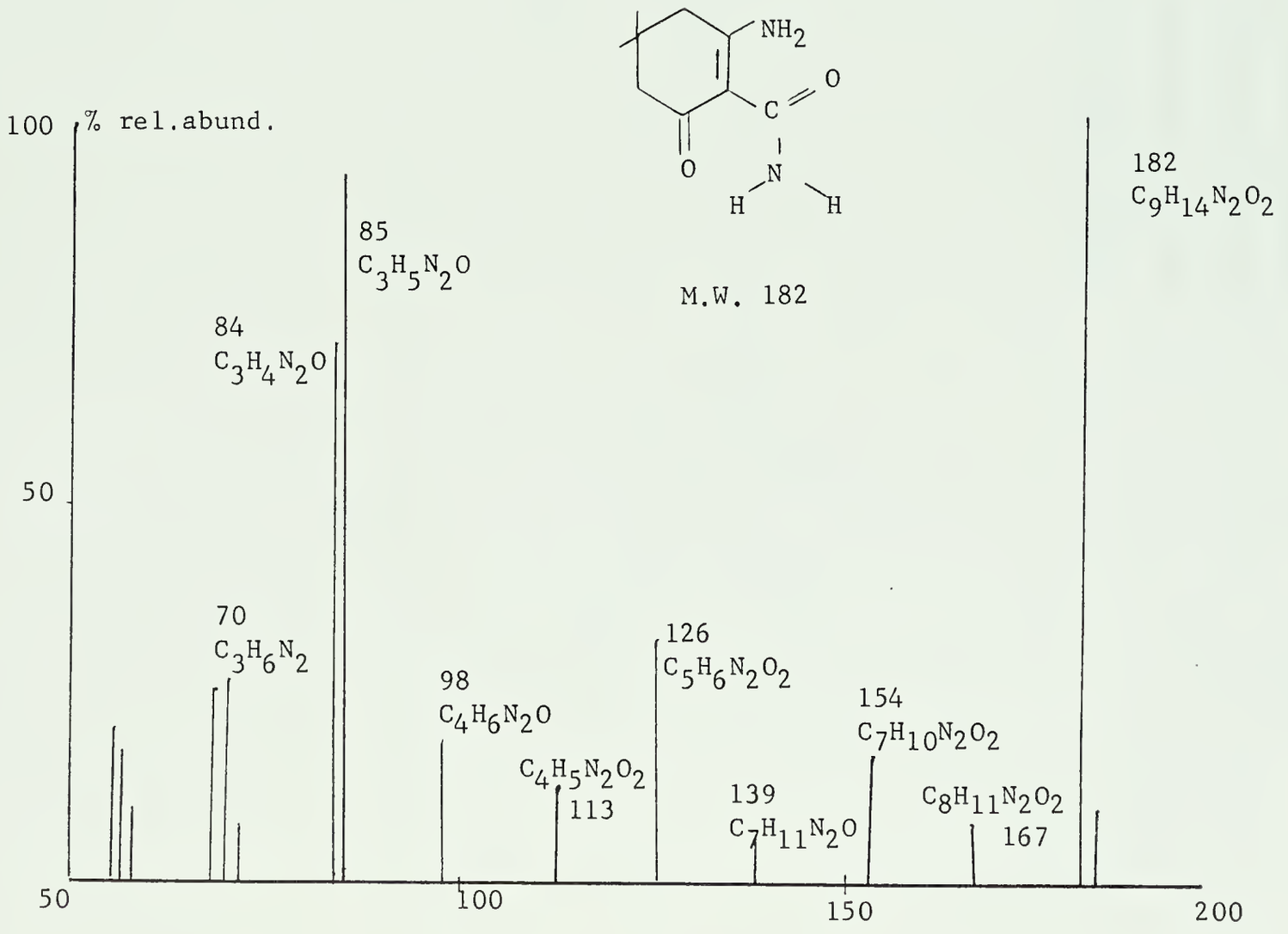
$C_5H_6N_2O_2$ m/e 126

$C_4H_6N_2O$ m/e 98



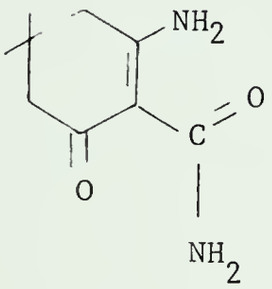
$C_3H_4N_2O$ m/e 84

Figure 2. Mass Spectrum of 3-amino-2-carboxamido-5,5-dimethyl-2-cyclohexen-1-one (153).

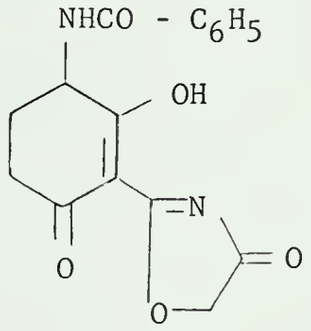


structural assignment was the four-proton methylene singlet at δ 2.4 in the nmr spectrum. There was no evidence of any resolution of the two methylene groups in CD_3COCD_3, $CDCl_3$, C_5D_5N, or CF_3COOD.

When α-chloroacetyl isocyanate was allowed to react with 4-benzamido-cyclohexane-1,3-dione (114) a compound related to 149 was obtained, namely compound 154.



153



154

CONCLUSION

The ultimate success of the last scheme proposed toward the synthesis of tetracycline-like A-ring systems might depend on C-2 carboxamidation using established isocyanates such as acetyl isocyanate or chlorosulfonyl isocyanate on 4-benzamido-cyclohexane-1,3-dione. Provided that the carboxamidation occurred as expected, isolation of the final product would still depend on the success of the reaction for removing the benzoyl protective group. In order to achieve this end reaction parameters would have to be established initially on some readily accessible model compounds. Synthetic schemes which involve basic or acidic hydrolytic conditions on 1,3-cyclohexanedione system must be avoided as our present studies and those of Muxfeldt clearly point to their problems and disadvantages. The oxazolin-4-one analogs 149 and 154 will be subjected to a broad pharmacologic screening in order to assess their potential as therapeutically useful compounds.

EXPERIMENTAL

Melting points were determined on a Thomas Hoover capillary melting point apparatus. All melting points are uncorrected. Infrared (ir) spectra were recorded on an Unicam SP 1000 Infrared Spectrophotometer and on a Perkin-Elmer 267 Grating Infrared Spectrophotometer. Ultraviolet (uv) spectra were recorded on an Unicam SP 1800 Ultraviolet Spectrophotometer. Nuclear magnetic resonance (nmr) spectra were taken on a Varian A-60 and a EM-360 60 MHz Spectrometer. Tetramethylsilane was used as an internal standard. Mass spectra were recorded at the Department of Chemistry, University of Alberta with an A.E.I. MS-12 or MS-50 mass spectrometer using the direct probe technique. Elemental analyses were determined at the Department of Chemistry and Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta.

The following abbreviations will be used in this section:

br = broad

d = doublet

m = multiplet

q = quartet

s = singlet

t = triplet

ϵ = molar absorptivity

Ethyl 2,4-di(1,2-dioxanyl)cyclohexanecarboxylate (128)

A stirred mixture of 4-carboethoxycyclohexane-1,3-dione (126) (18.5 g, 0.1 m), benzene (250 ml) ethylene glycol (100 g, 1.6 m) and p-toluenesulfonic acid (0.5 g) was heated to reflux for two days in a

round-bottom flask fitted with a Dean-Stark water separator. The benzene layer was separated and the glycol layer was extracted with diethyl ether (3 x 40 ml). The benzene and ether extracts were combined, washed with a 10% sodium carbonate solution and water respectively. The organic solvent was dried and evaporated to dryness under reduced pressure (20.3 g, 74%). An analytical sample was obtained through tlc (benzene:ethanol 9:1; silica gel).

$n_D^{24.4}$ 1.4826.

$ir_{(neat)}$: 2980, 2790, 1730 (ester carbonyl), 1450, 1370, 1180, 1080, 1030, 950 and 825 cm^{-1}.

$nmr_{(CDCl_3)}$: δ 1.30 (t,3, J = 6-7 Hz, $-OCH_2\underline{CH_3}$), 1.5-3.1 (m, 7, cyclohexane ring protons), 4.0-4.4 (m, 10, $-OC\underline{H_2}CH_3$ and $2(-OC\underline{H_2}C\underline{H_2}O-)$).

Anal. Calcd. for $C_{13}H_{20}O_6$: C, 57.34; H, 7.40.

Found: C, 57.77; H, 7.46.

2,4-Di(1,2-dioxanyl)cyclohexanecarbohydrazide (129)

Hydrazine hydrate (40 ml; 0.6 m) in anhydrous ethanol (20 ml) was stirred magnetically and heated to reflux. The diketal ester 128 (27.2 g; 0.1 m) in anhydrous ethanol (20 ml) was added dropwise over half an hour. The solution was refluxed for thirty hours, the ethanol was removed at reduced pressure and the remaining solution was evaporated to dryness under vacuo. The residual oil was dried in a vacuum dessicator for two days, and then triturated with anhydrous ethanol to give 25 g of the crude product. Recrystallization from ethanol afforded the title compound which melted at 115-116°.

$ir_{(disc)}$: 3400, 3300, 3200 (N-H); 1670 (amide carbonyl), 1510 (N-H bending), 1370, 1210, 1150, 1090, 1070, 1060, 1040, 950, 830, 800, and 740 cm^{-1}.

$nmr_{(CDCl_3)}$: δ 1.6-2.2 (m, 6, $3C\underline{H}_2$), 2.52 (t, 1, J=6 Hz, O=C($NHNH_2$)-$C\underline{H}$-), 3.68 (br, 2, O=C-$NHN\underline{H}_2$), 3.93 (d, 8, J=3 Hz 2(-$OC\underline{H}_2$ $C\underline{H}_2O$-)), 7.64 (br 1, O=C-$N\underline{H}NH_2$).

mass spectrum: $C_{11}H_{18}NO_3$, m/e calcd. 258.1211, meas. 258.1214 (53.8); 259(7.2), 199(13.7), 172(26.1), 141(50.8), 113(49.1), 99(98.7), 87(63.4), 86(45.8), 69(26.1), 55(100), m/e (%rel. abund.).

Ethyl 2,4-di(1,2-dioxanyl)-cyclohexylurethan (130)

The hydrazide 129 (2.2 g, 0.0085 m) dissolved in 6N HCl (3 ml), 5% acetic acid (5 ml) and ether (25 ml) was chilled to 0-5°, and was stirred magnetically. A solution of sodium nitrite (0.6 g in 1 ml water, 0.0085 m) was then added at such a rate that temperature did not rise above 10°. After addition was completed, the ether layer was separated and the aqueous solution was extracted four times with ether. The combined extract was washed twice with a saturated $NaHCO_3$ solution, twice with water, dried and filtered. To the filtrate was added an excess of anhydrous ethanol. The ether was removed by distillation and the resultant ethanolic solution was refluxed for four hours. The ethanol was evaporated under reduced pressure, and the viscous liquid residue solidified on cooling (1.85 g, 75%). Recrystallization from chloroform afforded the title compound, m.p. 124°.

$ir_{(disc)}$: 3340 (N-H stretching), 2980, 2790, 1720 (urethan carbonyl), 1545, 1330, 1250, 1150, 1080, 1060, 1030, 950 and 830 cm^{-1}.

$nmr_{(CDCl_3)}$: δ 1.26 (t, 3, J=7Hz, $-OCH_2C\underline{H}_3$), 3.7-4.3 (m, 10, 2(-$OC\underline{H}_2C\underline{H}_2O$-) and $-OC\underline{H}_2CH_3$), 4.8 (br, 1, $-N\underline{H}(CO_2C_2H_5)$).

mass spectrum: $C_{13}H_{21}NO_6$ m/e calcd. 287.
288(0.6), 287(1.5), 201(63.9), 187(14.1), 173(26.4), 159(27.2), 157(73.3), 100(29.2), 99(54.1), 87(70.7), 86(100), 73(33.5), 57(31.7), 55(27.7), m/e (% rel. abund.).

Anal calcd. for $C_{13}H_{21}NO_6$: C, 54.34; H, 7.36; N, 4.81.
Found: C, 53.99; H, 7.28; N, 4.92.

Ethyl 2-nitro-5-oxo-hexanoate (133)

To a stirred solution of ethyl nitroacetate (13.3 g, 0.1 m) and methyl vinyl ketone (7 g, 0.1 m) in THF (50 ml) at room temperature was added dropwise a catalytic amount of tri-n-butyl phosphine. The heat evolved was sufficient to bring the solution to a gentle reflux. After the solution had returned to room temperature (about two hours) methyl iodide was added to remove the phosphine. Direct distillation yielded ethyl 2-nitro-5-oxo-hexanoate (133). (16 g,78%), b.p. 134-138°, 3.5 mm, $n_D^{23.2}$ 1.4473.

$ir_{(neat)}$: 2980, 2960, 2940, 2910, 2870, 1750, 1720, 1560 (nitro stretching), 1370, 1260, 1170, 1020 and 860 cm^{-1}.

$nmr_{(CDCl_3)}$: δ 1.28 (t, 3, J= 7Hz, $-OCH_2\underline{CH_3}$), 2.14 (s, 3, $-COCH_3$), 4.25 (quartet, 2, J= 7HZ, $-OCH_2CH_3$), 5.26 (t, 1, $-CH(NO_2)CO_2C_2H_5$).

Anal. calcd. for $C_8H_{13}NO_5$: C, 47.28; H, 6.44; N, 6.89.
Found: C, 47.23; H, 6.40; N, 6.81.

Ethyl 2-nitro-5-(1,2-dioxanyl)-hexanoate (134)

A stirred mixture of ethyl 2-nitro-5-oxo-hexanoate (133) (15 g, 0.074 m) in benzene (160 ml), ethylene glycol (100 ml) and p-toluene-sulfonic acid (0.5 g) was heated to reflux under a Dean-Stark trap for 24 hours. The mixture was extracted with diethyl ether (3 x 80 ml),

washed once with a 10% sodium bicarbonate solution and once with water. The ether was dried, filtered and evaporated in vacuo. The resulting liquid was slowly and carefully vacuum distilled at temperature below 200° to yield a pale-yellow liquid (12 g, 68%). b.p. 112-116°, 2mm, $n_D^{20.0}$ 1.4547.

$ir_{(neat)}$: 2980, 2960, 2930, 2880, 1750, 1560 (nitro stretching), 1450, 1375, 1260, 1060, 1040, 950 and 860 cm^{-1}.

$nmr_{(CDCl_3)}$: δ 1.33 (t, 6, J=7HZ, terminal methyl and $-OCH_2\underline{CH_3}$), 3.96 (s, 4, $-OCH_2\underline{CH_2}O-$), 4.3(q, 2, $-OCH_2CH_3$), 5.28 (t, 1, J=7Hz, $-\underline{CH}(NO_2)CO_2C_2H_5$).

mass spectrum: 232.0820 ($M^+ - CH_3$) (14.9), $C_{10}H_{17}NO_6 - CH_3$ requires 232.0820, 99(14.8), 87(100), 86(24.3), m/e (% rel. abund.)

Anal. Calcd. for $C_{10}H_{17}NO_6$: C, 48.58; H, 6.93; N, 5.67.

Found: C, 49.64; H, 7.10; N, 5.78.

Ethyl 2-amino-5-(1,2-dioxanyl)-hexanoate (135)

Ethyl 2-nitro-5-(1,2-dioxanyl)-hexanoate (134) (20.3 g, 0.085 m) was mixed with 5 g of 10% palladium-on-charcoal in 95% ethanol (150ml). The nitro compound was hydrogenated under 1,500 psi at 50° for 10 hours. The catalyst was filtered, and the solvent was evaporated. The liquid residue was dissolved in benzene, dried and finally evaporated to give an oily liquid. (17 g, 93%). It solidified on standing for a few days at room temperature. An analytic sample had a m.p. of 212-214° (dec.).

$ir_{(disc)}$: 3200, 3080, 3030 (N-H stretchings), 2980, 2880, 1670, 1450, 1380, 1330, 1250, 1220, 1140, 1065, 1050, 950, and 870 cm^{-1}.

$nmr_{(CDCl_3)}$: δ 1.0-1.5 (m, 6, terminal methyl and $-OCH_2\underline{CH_3}$), 2.25 (s, 2, $-CH(\underline{NH_2})CO_2C_2H_5$), 3.90 (s, 4, $-OCH_2\underline{CH_2}O$), 4.17 (q, 2, J=7Hz, $-OCH_2CH_3$).

Anal. Calcd. for $C_{10}H_{19}NO_4$: C, 55.28; H, 8.81; N, 6.44.

Found: C, 55.27; H, 8.80; N, 6.32.

Ethyl 2-dimethylamino-5-oxo-hexanoate (137)

Ethyl 2-Amino-5-(1,2-dioxanyl)-hexanoate (2.17 g, 0.01m) in 95% ethanol (60 ml) was hydrogenated at 40 psi over 10% palladium-on-charcoal with an excess of formaldehyde (37% solution) for five hours. The catalyst was filtered and the solution evaporated under reduced pressure. The residue was hydrolyzed with 10% HCl (12ml) for six hours at room temperature. The resulting solution was made alkaline with dilute KOH, It was extracted thrice with hot chloroform. The combined extract was washed once with water and dried. Evaporation under reduced pressure gave 1.5 g (75% overall yield) of the desired compound in a fairly pure state as justified by tlc. (Methanol:Chloroform 1:9, silica gel).

$ir_{(neat)}$: 2950, 2930, 2860, 2820, 2780, 1710-1730 (strong, keto and ester carbonyls), 1450, 1360, 1170, 1160, and 1030 cm^{-1}.

$nmr_{(CDCl_3)}$: δ 1.28 (t, 3, J=7Hz, $-OCH_2\underline{CH_3}$), 2.13 (s, 3, $-COCH_3$), 2.30 (s, 6, $-N(CH_3)_2$), 3.10 (t, 1, J=7Hz, $-CH(NMe_2)(CO_2C_2H_5)$), 4.15 (q, 2, J=7Hz, $-OCH_2CH_3$).

Anal. calcd. for $C_{10}H_{19}NO_3$: C, 59.68; H, 9.51.

Found: C, 60.10; H, 9.66.

3-Ethoxy-6-dimethylamino-2-cyclohexen-1-one (138)

Sodium hydride (288 mg, 50% oil suspension, 2 equivalents) in benzene (15 ml) was stirred under dry nitrogen and heated to 40°. The dimethylamino keto ester I37 (603 mg) was then added dropwise. After addition was completed, the mixture was refluxed for 10 hours. The solid was collected by filtration and washed with benzene. It was dissolved in water and the solution acidified with 2N HCl solution. The resultant solution was evaporated to dryness in vacuo. The solid mass was extracted with ethanol and the solvent evaporated. The salt obtained was dissolved

in water and basified with dilute $NaHCO_3$ solution. The solution was extracted thrice with chloroform. Evaporation of the solvent under reduced pressure gave a liquid compound which was purified by tlc (chloroform-methanol 3:1).

$ir_{(neat)}$: 2980, 2930, 2860, 2820, 2780 (dimethylamino strethcings), 1650 (conjugated carbonyl), 1600 (double bond conjugated with carbonyl), 1375, 1235, 1200, 1170, 1035, 1020, 900, 850, 810 and 690 cm^{-1}.

$nmr_{(CDCl_3)}$: δ 1.33 (t, 3, J=7Hz, $-OCH_2\underline{CH_3}$), 2.40 (s, 6, $-N(\underline{CH_3})_2$), 3.85 (q, 2, J=7Hz, $-OCH_2\underline{CH_3}$), 5.28 (s, 1, olefinic proton).

mass spectrum: $C_{10}H_{17}NO_2$ requires m/e 183.1255, meas. 183.1255; 184, M+1 (0.71), 183(3.14), 140(32.58), 112(5.95), 84(11.27), 71(100). m/e (% rel. abund.).

Ethyl 2-benzamido-5-(1,2-dioxanyl)-hexanoate (139)

To a stirred solution of ethyl 2-amino-5-(1,2-dioxanyl)-hexanoate (17 g, 0.08 m) in pyridine (20 ml) and benzene (40 ml) was added dropwise benzoyl chloride (11.5 g 0.08m) in 5 ml of benzene. The resulting mixture was heated to gentle reflux for 30 minutes and was then poured into 200 ml of water. The benzene layer was separated and the aqueous solution was extracted twice with benzene. The combined benzene solution was washed with water and with 5% sodium carbonate solution and dried with anhydrous sodium sulfate. The drying agent was removed by filtration and the benzene was evaporated to a small volume (10 ml). Hexane (about 40 ml) was stirred into the mixture, and the benzoyl derivative crystallized out on standing. (13 g, 50.7%). Recrystallization from benzene and pentane gave a white solid, m.p. 87 - 88°.

$ir_{(disc)}$: 3340 (amide N-H), 3060 (aromatic C-H), 2970, 2880, 1740 (ester C=O), 1640 (amide C=O), 1510, 1490, 1370, 1210, 1190, 1060, 950, 860, 720, 690 cm^{-1}.

$nmr_{(CDCl_3)}$: δ 1.30 (t, 3, J=6.5Hz, $-OCH_2\underline{CH_3}$), 3.92 (s, 4, $-OCH_2\underline{CH_2}O-$), 4.22 (q, 2, J=7Hz, $OCH_2\underline{CH_3}$), 4.75 (t, 1, J=6.5Hz, $C\underline{H}$), 7.0-8.0 (m, 6, $C_6\underline{H_5}$, $N\underline{H}CO-$).

Anal. calcd. for $C_{17}H_{23}NO_5$: C, 63.53; H, 7.21; N, 4.36.

Found: C, 63.35; H, 7.27; N, 4.38.

The oxo compound 140 was obtained in quantitative yield from ethyl 2-benzamido-5-(1,2-dioxanyl)-hexanoate 139 by hydrolysis in dilute HCl at room temperature for several hours.

$nmr_{(CDCl_3)}$: δ 1.27 (t, 3, J=7Hz, $-OCH_2\underline{CH_3}$), 2.12 (s, 3, $-COCH_3$), 4.20 (q, 2, J=7Hz, $-OCH_2CH_3$), 4.76 (t, 1, J=6.5 Hz, $C\underline{H}$), 7.0-8.0 (m, 6, $C_6\underline{H_5}$, $N\underline{H}CO-$).

4-Benzamido-cyclohexane-1,3-dione (114)

To a stirred mixture of sodium hydride (2.4 g, 50% NaH suspension in oil, 0.05 m) in dry benzene (150 ml) under nitrogen was added dropwise a solution of ethyl 2-benzamido-5-oxo-hexanoate (7 g, 0.025m) in benzene (20 ml). The mixture was kept at 50-60° for an hour and brought to a gentle reflux for 5 hours. The solvent was filtered and the solid obtained was dissolved in water (150 ml) and acidified with dilute HCl. The solution was filtered and the precipitate collected. The filtrate was extracted twice with acetonitrile, dried and evaporated in vacuo. The solid so obtained was combined with that obtained from filtration. Recrystallization was effected in acetone to yield a white solid of m.p. 175-177° (dec.) (Lit.[78] m.p. 175-177°).

$ir_{(disc)}$: 3300 (amide N-H), 3060 (aromatic C-H), 2920, 2880, 2500-2700 (br, associated O-H), 1630-1590, 1520-1540, 1330, 1340, 1260, 1200, 920, 840, 710, 700 cm^{-1}.

$nmr_{(DMSO-d_6)}$: δ 4.65 (t, 1, J=7Hz, C<u>H</u>($NHCOC_6H_5$)),5.3 (s, 1, -C<u>H</u>=C(OH)-), 7.00-8.0 (m, 6, $C_6\underline{H}_5$, N<u>H</u>CO-), 8.36 (m, 1, -CH=C(O<u>H</u>)-).

mass spectrum: 231, $M^{+\cdot}$ (16.7), 203 (98), 185(7.6), 175(3.5), 122(95), 105(100), 77(99), m/e (% rel. abund.).Metastables at 178.5, 150.9, 168.5 and 73.3.

Anal. Calcd. for $C_{13}H_{13}NO_3$: C, 67.53; H, 5.66; N, 6.05.
Found: C, 67.58; H, 5.79; N, 5.80.

<u>3-Ethoxy-6-benzamido-2-cyclohexen-1-one (144)</u>

4-Benzamido-cyclohexane-1,3-dione (378 mg; 1.6 mM) and tetra-N-methyl-1,8-naphthalene diamine, "Proton-Sponge" (428 mg; 2.0 mM) were stirred in anhydrous methylene chloride (10 ml) under an atmosphere of dry nitrogen. To the clear solution was added triethyloxonium tetrafluoroborate (1.52 g; 8 mM) in methylene chloride (10 ml). Stirring was continued for four hours; afterwards the reaction mixture was treated with anhydrous methanol (20 ml) and stirred for ten hours. The solvent was evaporated in vacuo. The brown oil obtained was dissolved in ethyl acetate and stirred with an aqueous solution of sodium acetate for three hours. The organic layer was separated and the aqueous portion was extracted twice with ethyl acetate. The combined organic extracts was washed with brine once. Evaporation

of the solvent in vacuo gave a vicuous brown oil (165 mg; 39.8%).

$ir_{(neat)}$: 3300 (broad), 3060, 2970, 2930, 1715, 1640, (broad, intense), 1600(intense), 1575, 1540, 1530, 1485, 1380, 1330, 1230, 1200 (strong), 1100, 1035, 980, 920, 710, and 692 cm^{-1}.

$nmr_{(CDCl_3)}$: δ 1.20 (t, 3, J=7Hz, $-OCH_2\underline{C}H_3$), 3.50 (q, 2, J=7Hz, $-OC\underline{H}_2CH_3$), 4.50 (m, 1, $C\underline{H}(NHCOC_6H_5)$), 5.45 (s, 1, $-C\underline{H}=C(OC_2H_5)-$), 7.00-8.00 (m, 6, $C_6\underline{H}_5$, $N\underline{H}CO-$),

mass spectrum: $C_{15}H_{17}NO_3$ requires m/e 259.1204, meas. 259.1199. 260, M+1 (0.80), 259 (2.05), 245 (2.12), 203 (3.16), 154 (3.65), 138 (19.83), 112 (3.24), 106 (6.47), 105 (100), 98 (20.55), 97 (3.62), 84 (2.53), 77 (72.69), 68 (34.62); m/e (% rel. abund.).

α-Chloroacetyl isocyanate was prepared by the method of Speziale and Smith[93] and was further characterized as its ethyl urethan, $ClCH_2$-CONH-$CO_2C_2H_5$, m.p. 127.5-128°.

Anal. Calcd. for $C_5H_8NO_3Cl$: C, 36.27; H, 4.87; N, 8.46.

Found: C, 36.18; H, 4.80; N, 8.31.

3-hydroxy-5,5-dimethyl-2(2-oxazolin-4-oxo)-2-cyclohexen-1-one (149)

A solution of dimedone sodium enolate (1.4 g, 0.01m) and α-chloroacetyl isocyanate (2.4 g, 0.02m) in acetonitrile (30 ml) was refluxed for six hours. The solution was acidified with glacial acetic acid (1 ml) and evaporated in vacuo. The residue was stirred with water (10 ml), and acidified with dil.HCl until distinctly acidic. The acid solution was extracted with ethyl acetate (2X40 ml), washed with a saturated sodium chloride solution (30 ml), dried, filtered and evaporated under reduced pressure. The residue was

triturated with petroleum ether to give a solid precipitate (1.6 g; 73%). Recrystallization from acetone yield a yellow, puff-like solid, m.p. 227-229°.

uv: EtOH λ_{max} 236 nm (log ϵ 3.95), 290 nm (log ϵ 4.14).

EtOH,NaOH λ_{max} 258 nm (log ϵ 4.04), 308 nm (log ϵ 4.22).

$ir_{(disc)}$: 3140, 2950, 1770, 1670, 1600, 1515, 1430, 1320, 1200, 1060, 980, 820, 750, 680, 620 cm^{-1}.

$nmr_{(CDCl_3)}$: δ 1.08(s, 6, gem-dimethyls), 2.48 Id, 4, J=8 Hz, $\underline{CH_2}$), 4.85 (s, 2, $\underline{CH_2}$), 12.5 (s, br, 1, $\underline{OH}$).

mass spectrum: 223.0849, $C_{11}H_{13}NO_4$ requires 223.0843, 224(9.8), 223(63.7), 195(6.4), 167(33.5), 139(7.4), 126(45), 111(100), 83(61.8), 67(23.0); m/e(%rel.abund.).

3-Amino-2-carboxamido-5,5-dimethyl-2-cyclohexen-1-one (153)

149 (223 mg; 1 mM) was dissolved in 5 ml of saturated ammonical methanol. After standing at room temperature for 24 hours, the solvent was evaporated in vacuo. The residue was dissolved in acetone from which colorless crystals crystallized out (115 mg; 63%) m.p. 188-189° (dec).

uv: EtOH λ_{max} 260 nm, ϵ = 64.6.

$ir_{(KBr)}$: 3400, 3300, 3200, 3140, 2950, 2930, 2860, 1630(intense), 1600-1580 (br, intense), 1470, 1390, 1383, 1378, 1370, 1330, 1280, 1080, 760, 635 cm^{-1}.

nmr$_{(CD_3COCD_3)}$: δ 1.04 (s, 6, gem-dimethyls), 2.40 (s, 4, CH_2), 3.07 (s, 2, $-NH_2$), 6.77 (br, 1, -CONH-), 10.57 (br, 1, -CONH-).

ms: 182.1049, $C_9H_{14}N_2O_2$ requires 182.1054, 183(10.36), 182(100), 167(8.29), 154(17.44), 139(6.31), 126(32.04), 113(13.20), 98(17.60), 85(93.92), 84(71.66), 71(8.40), 70(27.48), 68(25.98), 57(10.41), 56(17.15), 55(21.71).

4-Benzamido-3-hydroxy-2(2-oxazolin-4-oxo)-2 cyclohexene-1-one (154)

4-Benzamido-cyclohexane-1,3-dione (114) (462 mg, 2 mM) was stirred for an hour with a 2N ethanolic solution of sodium ethoxide (1 ml; 2 mM). Ethanol was evaporated in vacuo and the resulting sodium enolate of 114 was dried in vacuo overnight. To the dried sodium enolate in acetonitrile (15 ml) under an atmosphere of dry nitrogen was added α-chloroacetyl isocyanate (480 mg; 4 mM) from a syringe. The mixture was stirred for an hour an room temperature and later refluxed for eight hours. The mixture was diluted with water (5 ml) and acidified to pH~2 with dilute HCl; and extracted with ethyl acetate-acetonitrile (1:2). The combined extract was washed with brine, dried over anhydrous sodium sulfate and evaporated in vacuo. The brown liquid residue after evaporation was triturated with chloroform, thereupon a precipitate appeared and was collected by filtration (305 mg; 48.5%).

ir$_{(disc)}$: 3300 (medium), 3060, 2960, 2900, 1790 (intense), 1680 (intense), 1625 (br, intense), 1600 (strong), 1580, 1520 (br, intense), 1440, 1400, 1350, 1306 (strong), 1205, 1100, 1060, 1045, 985, 960, 940, 860, 815, 800, 765, 715 and 680 cm^{-1}.

$nmr_{(DMSO\text{-}d_6)}$: δ 5.00 (s, 2, $C\underline{H}_2$), 7.00-8.00 (m, 6, $C_6\underline{H}_5$, $N\underline{H}CO$-), 8.60 (m, 1, $O\underline{H}$).

mass spectrum: $C_{16}H_{14}N_2O_5$ requires m/e 314.0902, meas. 314.0908. 315, M + 1 (2.65), 314(13.07), 209(15.76), 194(5.31), 193(53.36), 181(8.85), 167(5.34), 161(5.13), 154(3.22), 151(4.89), 149(10.69), 126(10.38), 125(3.53), 123(.752), 122(5.04), 106(8.19), 105(100), 84(5.27), 77(47.76), 68(5.80), 67(4.50).

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